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OF
PROGRAM ACTIVITIES

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U.S. NATIONAL CANCER INSTITUTE

ANNUAL REPORT of program activities

July 1, 1967 through June 30, 1968

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NATIONAL CANCER INSTITUTE
July 1, 1967 - June 30, 1968

Introduction

The past year was a period for the National Cancer Institute during which a number of outstanding developments took place, highlighted by: a visit of the President of the United States to Bethesda for a briefing by the Directors of this and others of the National Institutes of Health on progress in medical research; the establishment of a Lung Cancer Task Force, and a major reorganization within the Office of the Director. As reported to the President, research efforts were relentlessly pursued on a number of fronts.

Approximately two-thirds of the Institute's appropriation was allocated about equally between programmed research on prevention and treatment. Studies designed to identify different causes of cancer and eliminate them from the human environment continued to move along two main thrusts: research on viruses as highly suspected causative agents, and investigation of chemicals that are being introduced into the working and general environments at fantastic rates. Also under study were the cancer-causing effects of radiation in different forms, and individual conditions predisposing to cancer, such as hereditary susceptibility, hormonal imbalance and lowered resistance.

Research for the improvement of treatment was directed mainly toward the development of new or improved drugs and their utilization for maximum efficacy. In at least half of all cancer cases, the disease has spread to an extent precluding effective surgery or radiation by the time the victim seeks medical aid. The only real hope for such patients is drug treatment that can reach and destroy cancer cells wherever they may be in the body.

These programmed activities made special use of increasing knowledge of the cancer process gained through continuing fundamental research. Approximately one-sixth of the Institute's appropriation was devoted to this unprogrammed inquiry into the general characteristics and bases for normal and abnormal cell development, leading to improved understanding of the nature of cancer. They also made use of knowledge of the impact of cancer, gained through epidemiological and statistical studies of the distribution of different forms of cancer in this and other countries and the resulting economic burden.

A number of task forces continued to work actively on various specific disease problems. The contributions of the Acute Leukemia Task Force are particularly noteworthy and have materially strengthened and speeded the Institute's efforts to direct effective attacks on the problems of acute leukemia. The Breast Cancer Task Force, organized more recently, began to mount an intensive study of cause and treatment with emphasis on hormonal factors involved in this leading cause of cancer death in women. Most recently formed was the Lung Cancer Task Force, of which more below.

Visit of the President

President Johnson's visit to the National Institutes of Health on July 21, 1967 was for the purpose of seeing at first hand how American tax dollars are being spent for the improvement of the Nation's health. The Director, NCI, while not minimizing the serious cancer problem confronting the U.S. population, made it plain to the Chief Executive that there is a climate of hope in the cancer research community. He said, "We have had some success already, and I expect within a few years to see the cancer death rate turn downward through prevention as well as cures."

The President was given a published report, "The Advancement of Knowledge for the Nation's Health," to which NCI staff contributed a segment of some 25 pages.

Lung Cancer Task Force

At the direction of the President, as expressed in his Health Message to the Congress, a 10-member Lung Cancer Task Force made up of physicians and scientists was established under the aegis of the Institute. The Director, NCI, who was named chairman of the Task Force, announced its establishment at a press briefing held in the Department of Health, Education, and Welfare.

The group has held meetings for orientation and formulation of general guidelines for a diverse attack on the lung cancer problem. The Task Force will concentrate on research for the development of a less hazardous cigarette, identification and elimination of environmental carcinogens, and improvement through development of better diagnostic and therapeutic methods of the present low lung cancer cure rate which now lies between five and ten percent.

Reorganization Items

A reorganization of the Office of the Associate Director for Program went into effect to meet increasing demands from both National Cancer Institute program leaders and outside institutions for assistance in planning cancer training and research.

The new organization was designed to facilitate planning on a continuous basis, not only for single program efforts but coordinated research programs, both intramural and extramural.

Heading the new group, Dr. Jesse L. Steinfeld was named Associate Director for Program. Prior to this appointment, Dr. Steinfeld had served at the University of Southern California as Associate Professor of Medicine and Head of the Cancer Chemotherapy Program. In his new position he works closely with the Director, NCI, in coordinating the major Institute programs, and with members of the Institute's top extramural advisory committees on major problems and policy matters.

Named as Deputy Associate Director was Louis M. Carrese, Program Planning Officer since 1962 in the Office of the Associate Director. Mr. Carrese works with senior Institute staff in formulating research plans for segments of Institute program. He also reviews major components of Institute programs and outlines methods by which intramural and contracted research plans may be integrated more effectively.

The Program area was divided into two branches and a Scientific and Technical Information Office.

The Program Analysis and Formulation Branch, directed by Dr. Robert E. Greenfield, was established to outline major needs for research programs to solve specific problems in the etiology, prevention, diagnosis, treatment, and prognosis of cancer in man.

Drs. Michael Klein and Abraham Cantarow were named to assist Dr. Greenfield as Senior Research Planning Officers. In this capacity they provide guidance in the analysis, formulation and planning of integrated, comprehensive research programs.

The other Branch, Systems and Operations Planning, was initiated for the development and application of systems and operations planning techniques to biomedical research. Mr. Carrese was named Acting Chief and Richard A. Terselic was appointed Systems Analysis and Planning Officer for the Branch.

Dr. John H. Schneider was appointed Head of the Scientific and Technical Information Office. Among other activities he develops methods for improving scientific communication by designing and conducting an experiment in the Selective Dissemination of Information in cancer research. Mrs. Delores Bryla, a statistician in the Scientific and Technical Information Office, continued to provide answers to routine and special requests for scientific information, and to gather data for the planning of the Institute budget.

Other Reorganization Items. In the Baltimore Cancer Research Center, two section-level organizations were established: the Biochemistry Section, and the Immunology and Cell Biology Section. Three Services--Medical, Neurosurgical, and Radiation Therapy--were established in the Clinical Branch at Baltimore.

The Special Cancer Survey Section was established in the Biometry Branch, Demography, Etiology Area. Other sections making up the Branch are Demography, End Results, and Mathematical Statistics and Applied Mathematics.

Several organizational changes took place in the Viral Oncology Area, Etiology. In the Office of the Associate Director for Viral Oncology:

The Laboratory Aids Unit was abolished.

The Office of the Scientific Coordinator in Viral Oncology was established.

Three sections were established: Program Analysis and Communications, Program Resources and Logistics, and Research Support. A fourth section--the

Biohazards Control and Containment Section--was transferred to this Office from the Office of the Scientific Director for Etiology.

The Viral Leukemia Section was transferred from the Viral Biology Branch to the Viral Leukemia and Lymphoma Branch.

In the Viral Carcinogenesis Branch there were abolished the Primate Holding and Experimental Facility Section, the Viral Resources and Repository Section, and the Information Unit, Office of the Chief. Subsequently there were also abolished the Sero-Epidemiology Section and the Diagnostic Virology Section. Established were the Ecology and Epidemiology Section, and the Solid Tumor-Virus Section.

In the Metabolism Branch, General Laboratories and Clinics, there was established an Immunophysiology Section.

The Tumor-Host Relations Section, Laboratory of Biochemistry, General Laboratories and Clinics, was retitled Biosynthesis Section.

The Cytodiagnosis Service, Pathologic Anatomy Branch, General Laboratories and Clinics, was changed to Cytopathology Service.

Chemotherapy

Activity in the Chemotherapy area during the year was concentrated on the pursuit of two general objectives: 1) to identify in the more than three million known chemical compounds those chemicals that will best control cancer, and 2) to study the best ways to use the effective drugs in man.

A corollary to (1) is the objective to find chemical compounds now unknown but existing in nature--in bacteria, plants, and animals. A second corollary is to find out enough about the antitumor activity of the known effective drugs in animals and man, that it becomes possible to relate chemical structure with the desired antitumor activity. This growing knowledge will then enable the chemist to custom tailor new drugs to produce the desired effects.

An analysis of the first ten years' experience in the Institute's Chemotherapy program brought about the decision to change the primary animal tumor system screen, which now consists of L1210 leukemia in the mouse and Walker 256 in the rat. The new primary screen requires only one-third as much material as the old one and, for strongly positive compounds, permits much earlier decisions. It achieves a thousandfold concentration of most of the probable active compounds and permits rapid pursuit of highly active chemicals.

A new modality is assuming increasing importance in the decision to go for full clinical trial of selected compounds, namely, the demonstration by the methods of Skipper and Bruce, of exploitable selective toxicity for the tumor. This has stimulated an extraordinary amount of research on selective toxicity, and conceptual and technical advances are occurring rapidly. Quantitative systems permit discrimination among the myriad of agents, dosage schedules, and drug combinations, in favor of high selectivity for tumor cell kill. These promising leads are now under test in the clinic.

With regard to objective (2) of the Chemotherapy program--the study of the best ways to use the effective drugs in man--the problem goes right to the nature of the cancer cell: why it is that a cancer cell multiplies without regard to the rules of the body. The sensitivity of cancers to drugs is somehow related to the rate at which they grow. Chemical control has advanced remarkably for the rapidly-growing tumors--choriocarcinoma, the acute leukemias, Hodgkin's disease, Wilms's tumor. It seems likely that application of present knowledge will bring about almost complete control of these diseases. As this sensitivity to drugs has been studied on a fundamental level, it has been discovered that the biologic situation (especially the fraction of tumor cells dividing at any one time) in the slow-growing tumors is entirely different from the more rapidly growing tumors. The new knowledge must now constitute the basis for developing first in animal systems and then in man effective methods for the treatment of slow-growing tumors.

General Laboratories and Clinics

Within the Institute organization, numerous highly qualified independent investigators continued to be engaged full-time on a wide variety of problems in the cancer field. While many decisions as to the selection of research topics and approaches are left to the individual, these research efforts are yet more interrelated than those in the grant-supported area, since decisions on recruitment, promotions, budget, and space allocations must be made within the total organizational framework. Budgeting too is on the basis not of the individual project, but on the broader basis of a branch or laboratory, generally organized around scientific disciplines or clinical specialties. Moreover, in the clinical part of this area, provision of sound medical care to patients participating in investigations takes priority over all other considerations.

This group continues to be a major source for developing mature cancer investigators and program leaders within the Government and outside it. Because of the full-time focus on cancer in a setting in which nearly all aspects of cancer are dealt with on a continuing basis, and in which a wide variety of other biomedical capabilities are available, the opportunities for development of cancer-research capability within the Institute are unique.

The existence of the in-house programs and responsibility for their operations assure continual inputs to staff administering Federal cancer research programs. This input cannot be provided by such other means as intermittent advisory committee meetings or reliance upon scientific and medical literature alone. The in-house scientists of the NCI have made major contributions to accomplishments in cancer research and continue to do so. From these scientists have come, for example, many of the major advances in virology and therapy, particularly in leukemia and choriocarcinoma. Their findings have provided the knowledge, models, and basis for the programmed efforts in etiology and therapy.

Virus Programs

The highlight of the viral oncology program of the Etiology area during the past year was the launching of the Solid Tumor Virus Program Segment (STVP) of the Special Virus Leukemia Program (SVLP). The new segment was largely planned in fiscal 1967 and began getting under way in fiscal 1968. The SVLP had its beginnings in fiscal year 1965.

The establishment of the SVLP was based on the conviction that there exists sufficient knowledge and technical capability to plan and implement an intensified and coordinated program in leukemia research. This program includes studies aimed at determining whether viruses comparable to those known to cause leukemia in many laboratory and domestic animals are causative agents of human forms of the disease and, if so, developing effective means for their prevention or control.

A number of laboratories within the Etiology area provide the in-house intellectual and technical guidance and thrust for the SVLP. Contract projects within this program are chosen and awarded on the basis of technical and intellectual excellence, need, and relevance to specific program objectives. From studies conducted within the framework of an overall program plan several significant facts and leads have emerged during the past year.

For example, it is now apparent that virus-like particles of at least two different types can be detected in--and in some cases isolated from--tissues of leukemia and lymphoma patients around the world. These are a herpes-type virus (HTV) similar to those causing "cold sores" and a C-type which resembles Shope viruses causing animal leukemias.

The herpes-type has been the one virus most persistently detected. At least 42 of the more than 60 tissue cultures established from cells of patients with leukemia and lymphoma contain the HTV. In addition, this virus has been found, prior to culture, in tissues of a few leukemia patients, in representatives of many animal species, in people with non-cancerous diseases, and in "normal" healthy individuals. The widespread distribution of this virus suggests that, if it is related to leukemia or lymphoma, a "normal" non-diseased carrier may be a source of infection for highly susceptible individuals.

Work is accelerating, too, on the C-type particles. Collaborative studies conducted by clinical and laboratory investigators during the past year have made it possible to collect very large fractions of blood suspected of containing the particles. This material will be used in comparative studies of the human C-type virus, the mouse and chicken leukemia viruses, and the herpes-type virus.

Attempts to transmit human leukemia to laboratory animals have not yet been successful although materials from patients have been inoculated into more than 600 newborn primates and into large numbers of dogs, cats, mice, and hamsters. The herpes-type virus associated with leukemia and lymphoma cell cultures has shown some evidence of infectivity among hamsters and kittens.

Comparative studies on African and American patients with lymphoma have been expanded and strengthened during the past year through establishment of close working relationships between key scientists in this country and Africa. In Africa, where Burkitt's lymphoma occurs in children at an incidence higher than that for all other forms of cancer, certain regions might well be used for field trials of candidate leukemia/lymphoma preventatives.

The Solid Tumor Virus Program Segment (STVP), established during the past year as one of the eight working groups of the Special Virus Leukemia Program, shares many of the problems and many of the successes of the older program. The common denominator to virtually all management problems is time. The virus cancer program of the NCI now includes more than 90 contracts, each of which must be managed individually, as well as integrated with the total program. A very high level has been attained in the coordination of similar scientific activities conducted by investigators in Government, university, and commercial laboratories. However, this effective scientific management of contracted research requires an extraordinary amount of time by project officers and program managers, frequently provided at the expense of intramural research activities.

Chemical Carcinogenesis Program

Many programmed studies in carcinogenesis at the Institute are concerned with the roles of chemical agents in the induction of cancers in man, improving the means for identifying such agents, and learning more of the ways in which cancer-causing chemicals induce cancers. Greatest emphasis in both contracted and in-house research has been put on those factors which may constitute carcinogenic hazards for large segments of the human population. However, studies of agents affecting smaller groups are also of great importance. Secondary factors in the causation of cancers are also under study.

Possible sources of external cancer-causing chemicals may be broadly categorized as contaminated food, tobacco, polluted air, pesticides, and pharmaceutical agents. NCI carcinogenesis studies are going forward in all these areas.

Public Information and Education

During the year, the National Advisory Cancer Council issued "Progress Against Cancer 1967," its second report to the public, following the publication of its initial report, "Progress Against Cancer 1966." As was the case in 1966, last year's booklet was prepared by the Institute's Research Information Branch with review and approval by the Council. The 1967 publication dealt largely with the state of the art in virus-cancer research. The Council has also authorized a third report, to be issued in 1968, dealing with the general subject of cancer chemotherapy; and a fourth, the following year on chemical carcinogenesis.

The Institute arranged a contract with National Educational Television for the production of a two-part program on cancer chemotherapy research. The color films, each running 30 minutes, were scheduled for broadcast from February through August 1968 on the 133-station nationwide NET network, to an estimated 18,000,000 viewers. Entitled "Drugs Against Cancer" (Part I--"The Search;" Part II--"The Battle in the Cell"), the films will also be available for loan by NCI and NET to schools and interested groups.

Staff Changes, Honors and Awards

Dr. Ian A. Mitchell, former Associate Director for Planning and Analysis, Etiology, was appointed an Assistant Director of the Institute.

Dr. Howard B. Andervont retired from his position in the Laboratory of Biology and as Scientific Editor of the *Journal of the National Cancer Institute*. The June issue of the *Journal* was dedicated to him.

Dr. Thaddeus J. Domanski, former Scientist Administrator, Research Grants Branch, NIGMS, was appointed Program Director for Chemical Carcinogenesis, Extramural Activities. In addition, Dr. Domanski was named to serve as Chief, Awards Review and Technical Branch.

Public Health Service Commendation Medals were presented to Dr. Seymour M. Perry, Associate Scientific Director for Clinical Trials; Dr. Louis B. Thomas, Head, Surgical Pathology and Post Mortem Service; Dr. Paul Carbone, Head, Solid Tumor Service; Dr. Richard A. Tjalma, Head, Epizoology Section; and Mr. Robert S. Runkle, Vice-Chairman of the Biohazards Control and Containment Segment, Special Virus-Leukemia Program.

Dr. Frank J. Rauscher, Jr., Associate Scientific Director for Viral Oncology, was selected as one of the annual winners of the Arthur S. Flemming Award for outstanding government service.

Dr. John T. Kalberer, Jr., a former Grants Associate, was appointed a Special Assistant in the office of the Associate Director for Extramural Activities.

Dr. Harold P. Morris, a member of the research staff of the Institute since its founding, retired from his post in the Laboratory of Biochemistry.

Nineteen leading pathologists from Europe, Africa and North America met at the Institute in October to define the pathologic features of Burkitt's tumor and determine its place in a new classification of the lymphomas. The group was brought together by the World Health Organization. Dr. Humberto Torloni, Medical Officer of the Cancer Unit, WHO, served as secretary for the conference.

The NCI was host to a 5-day symposium co-sponsored by the National Science Foundation and the Japan Society for the Promotion of Science, November 27-December 1. The subject was the Pathology, Classification and Nomenclature of Leukemia, Malignant Lymphomas and Other Neoplastic Diseases of the Hematopoietic System.

"Progress in Clinical Cancer," Volume III, edited by Irving M. Ariel, M.D., was dedicated to Dr. John R. Heller, Special Consultant on International Programs.

William Z. Penland, Jr., former Staff Engineer for the biohazards segment of the Special Virus-Leukemia Program, was named Staff Engineer in the clinical trials area of the Chemotherapy Program.

Dr. Kurt Salomon resigned as Scientific Specialist, Awards Review and Technical Administration Branch, to accept an appointment as Professor and Head of a new Department of Radiation Biology and Radiotherapeutics at the Medical College of the University of Lagos, Nigeria.

Dr. Beverly Peterkofsky, guest worker in the Laboratory of Physiology, was awarded a 5-year Established Investigatorship by the American Heart Association to study factors controlling protein synthesis during embryonic development.

A scientific paper, "Morphological and Biochemical Studies of SV40 Transformed Prostatic Tissue," by Drs. Elwin E. Fraley and David F. Paulson of the Surgery Branch, won the first prize for research of the American Urological Association. It was the third time in four years that members of the Branch have won this prize.

Dr. C. Gordon Zubrod, Scientific Director for Chemotherapy, was awarded the Bicentennial Medal of the College of Physicians and Surgeons of Columbia University for his achievements in the field of Internal Medicine.

Miss Hilda Wexler, of the Surgery Branch, received the Edward F. Bartelt Award of the District of Columbia Division, American Cancer Society, for outstanding service in the cause of cancer control by a Federal employee.

Dr. Alfred S. Ketcham, Chief of the Surgery Branch, was awarded the Public Health Service Meritorious Service Medal.

Dr. T. Phillip Waalkes, Special Assistant to the Associate Scientific Director for Clinical Trials, was temporarily assigned to the Food and Drug Administration, to serve as Director for Clinical Research of an FDA antibiotic project.

Dr. Lloyd W. Law, Head, Tissue Culture Section, Laboratory of Biology, and President of the American Association for Cancer Research, delivered the Presidential Address at the 59th Annual Meeting of the Association. His title: "Studies of the Significance of Tumor Antigens in the Induction and Repression of Neoplastic Disease."

Dr. William W. Payne, Deputy Director of the Division of Environmental Health Sciences, NIH, was awarded the Public Health Service Commendation Medal in recognition of his services while a member of the Etiology staff, NCI.

Dr. Mearl F. Stanton of the Laboratory of Pathology was appointed Scientific Editor of the Journal of the National Cancer Institute.

ANNUAL REPORT OF THE CLINICAL DIRECTOR
NATIONAL CANCER INSTITUTE

July 1, 1967 through June 30, 1968

The Diagnostic Research Committee has for sometime recognized that there was considerable limitations in our present capability for the early detection and early diagnosis of cancer, and this can be considered a gap area. Neither the committee nor the staff have been able to find a means for closing this gap and this must remain a problem for the future. It is an important clinical problem, but there are few significant new leads.

This year has been the first year in which the Clinical Research Committee has functioned. This Committee is composed of the Clinical Branch Chiefs and Clinical Section Heads. It meets fairly frequently and has considered a number of research protocols involving patients. This Committee has not constituted an impediment to the carrying out of clinical research and, from time to time, its discussions with an investigator have proven to be helpful in the design and execution of studies.

The principal problem for the future, is to create an intensive care unit that would be concerned with reverse isolation. Our facilities for carrying out this type of study are limited and the need is here.

Program Activities Report
Fiscal Year 1967-68
Editorial Office of the National Cancer Institute

The Board of Editors reviewed 990 items for publication in various journals. Those not destined for the Journal of the National Cancer Institute constituted 469 manuscripts and 240 abstracts and book reviews.

Of 281 manuscripts submitted to the Journal of the National Cancer Institute, 49 were by NCI authors: 31, accepted; 8 rejected; 10, pending. Authors from other research institutions submitted 232: 108 accepted; 56, rejected; 4, withdrawn; 64 pending. Of these, 69 were received from other countries.

Volumes 38 and 39 of JNCI made 2,272 double-column pages.

Four NCI monographs totaling 1,381 pages were published: #25 Tumors of the Alimentary Tract in Africans; #26 Decennial Review Conference on Cell Tissue and Organ Culture; #27 International Symposium Enzymatic Aspects of Metabolic Regulation; and #28 Toward a Less Harmful Cigarette.

ANNUAL REPORT
OFFICE OF THE ASSOCIATE DIRECTOR FOR PROGRAM
NATIONAL CANCER INSTITUTE
July 1, 1967 - June 30, 1968

FY 1968 Activities of the Office of the Associate Director for Program (OADP)

During the past year the Office of the Associate Director for Program has undergone a series of organizational changes. In order more adequately to respond to the increasing demands from both internal program areas and outside institutions for assistance in research planning activities, two branches were established within the OADP; i.e., Systems and Operations Planning Branch, and Program Analysis and Formulation Branch, with a Deputy Associate Director for Program responsible for their direct supervision and coordination, and the general supervision under the Associate Director for Program. The reorganization has maintained the Office of Scientific and Technical Information and the Special Advisor to the ADP within the OADP. Shortly after the beginning of calendar year 1968 Dr. Jesse L. Steinfeld was appointed Associate Director for Program, to fill the position vacated by Dr. Carl G. Baker who assumed the duties of Scientific Director for Etiology. In addition, Louis M. Carrese, Program Planning Officer, NCI, was appointed Deputy Associate Director for Program and Chief of the Systems Operations and Planning Branch. Dr. Robert E. Greenfield, Research Planning Officer, NCI, was appointed Chief of the Program Analysis and Formulation Branch. Dr. John H. Schneider was named Scientific and Technical Information Officer for the Institute.

I. New Planning Activities

Formulation of initial plans for a program in lung cancer, utilizing the Convergence Technique, was completed and plans were presented to the Lung Cancer Task Force and to the National Advisory Cancer Council for approval during the fall, 1967. In addition to the planning activities performed in conjunction with NCI staff, discussions were held with other organizations interested in applying the Convergence Technique to research. These included: the U.S. Office of Education in the formulation of a program intended to expand the organized body of knowledge concerning the reading process in adolescents; NICHD concerning a major study of birth control techniques; the Bureau of Disease Prevention and Environmental Control in an R&D program in this area; the World Health Organization and the International Agency for Research on Cancer at Lyon in general program planning and organization.

During FY 1969 it is expected that major program planning efforts will be initiated in the areas of DNA virus research and chemical carcinogenesis. Further development of the Lung Cancer Program plan is also expected.

II. Initial Discussions and Studies Related to Development of an Institute Strategy Statement

At the request of the Director, NCI, the OADP staff has engaged in discussions and has begun to explore alternative positions that the Institute may take on questions of research and administrative strategy. No completion date has been projected for this effort. Preliminary discussions have been held and are continuing on the following topics: current and future research objectives of the NCI relative to its stated mission; needs and obstacles to research in cancer; resource needs for cancer including professional manpower; research personnel policies and climate for research; review and evaluation mechanisms for research and its administration; mechanisms of support for research; communication of information; and optimal organizational arrangements for the conduct of research.

III. Follow-up Planning

Work has continued in supporting the on-going evaluation of progress in the Special Virus-Leukemia Program, utilizing monitoring and reporting techniques formulated during the preceding year. Also, similar support is being provided the CCNSC in developing more effective systems and procedures for monitoring the progress of drugs through the Drug Development Flow.

IV. Revision and Updating of Program Plans

A major program plan revision was completed during FY 1968 on the Drug Development Flow of the Cancer Chemotherapy Program. This revision required several man-months of effort, involved the participation of most of the senior staff of the chemotherapy area, and included the development of a comprehensive decision network for all key program decisions. The revised plan is now being implemented.

V. Other Activities

Members of the staff have also participated actively in the deliberations of the Scientific Directorate, in the deliberations of the Planning Subcommittee and the Subcommittee on Etiology and Prevention of the NACC and in developing supporting informational materials for the NACC. Further, the ADP and DADP have personally engaged in the conduct of several studies on behalf of the Director, NCI, pertinent to specific operations within the Institute.

Activities of the Systems and Operations Planning Branch (SOPB)

The SOPB has undertaken several studies related to Institute operations problems and has participated, along with the PAFB, in other planning activities. Activities undertaken specifically by SOPB include:

I. Study of Innovations in Program Conduct and Management Approaches

To fully apprise the Officers and Advisory Groups of the National Cancer Institute of the relative merits of various alternatives to conducting and managing a significantly larger research program, SOPB has been engaged in studies of a number of differing approaches including:

#1 - Extended Applications of Program Contracting (complete),
#2 - Seeking Continued Improvement in the Operations of the NCI (draft now being reviewed),
#3 - A Discussion of the Utilization of Consortia for NCI Research (draft now being developed),
#4 - Extended Application of Cancer Research Centers (to be initiated during April, and to be completed by July, 1968).

The studies have been conducted through interviews with key NCI scientific and administrative officers, library research and inquiries to persons and information sources outside of NCI. Drafts of the studies are circulated to various individuals within the Institute for review and comment.

Upon completion, the series of studies and related recommendations will be presented to the Director, NCI and Scientific Directorate for review and decision concerning further dissemination.

II. Study of Scientific Reporting on Contracts in the Chemotherapy Program

At the request of the Scientific Director for Chemotherapy, the SOPB undertook an extensive study of scientific reporting procedures on Chemotherapy Program contracts. A single contract, considered sufficiently large and complex to be representative, was chosen as a case example for study. Project officers assigned to the contract were interviewed to determine if the procedures and approaches presently being used to monitor contract research activities were sufficiently effective. A report was prepared which includes:

1. A summary of findings and recommendations concerning potentially effective approaches to reporting and monitoring;
2. A training document for project officers entitled, "Program Monitoring, Observing and Reporting on Research/Development Contracts";
3. A proposed system for classifying the need for various types of reports on R/D contracts;
4. Proposed guidelines for preparation of contractor reports.

After review by members of the OADP staff, a draft of the full report was submitted to the Chief, CCNSC for comment prior to presentation to the Scientific Director for Chemotherapy.

It is felt that this report has general applicability beyond the Chemotherapy Program to all of NCI's contract operations.

III. Participation in an Institute-Wide Training Program for Project Officers

SOPB has participated with the Grants and Research Contracts Operation Branch in the planning of a training program to be conducted during May, 1968 for all NCI project officers. As a spin-off of the study of contract reporting in the Chemotherapy Program (Item II), SOPB was asked to prepare and present a lecture, as part of the training program, on the subject "Research Contract Monitoring". Preparations for the lecture have been completed.

Activities of the Program Analysis and Formulation Branch (PAFB)

The Program Analysis and Formulation Branch FY 1968 has responded to requests from different areas of the National Cancer Institute including the Director and the Assistant Directors, the Associate Director for Program, the Systems and Operations Planning Branch, OD, the National Advisory Cancer Council and various Scientific and Associate Directors. The specific contributions of the Branch are summarized for FY 1968 under the following headings:

I. Survey of Needs in Area of Chemical Carcinogenesis

At its September 1966 meeting, the National Advisory Cancer Council directed the staff to proceed with a survey of the area of chemical carcinogenesis with a view to (a) assessing needs for support of research and training in this field, and (b) developing a mechanism for evaluating, on a continuing basis, the effectiveness of the programs of the NCI in the several areas of cancer research.

During the past year, nine groups, comprising 110 participants, engaged in discussions of needs in this area, in attempts to identify important research objectives and the best means of attaining them. The practical objective was to develop a set of recommendations for consideration by the Council that would represent the consensus of a representative body of scientists actively engaged in research in chemical carcinogenesis or related fields. These discussions culminated in a final report to the Council in March, 1968. The Council unanimously approved the report in principle and indicated also its approval of this type of survey as a means of providing similar information on other areas of cancer research.

II. Completion of Outline of Cancer in Man

This system of classification of cancer research projects provides a goal-oriented framework (i.e., prevention, diagnosis, prognosis and treatment of cancer) against which discipline-based or phenomenon-based projects can be related. It is designed to complement the classification systems of Dr. Schneider and Mr. Canter, in current use.

Initial steps have been taken towards establishing liaison with other organizations with interests in cancer research, with a view to establishing a procedure for exchange of information on distribution of support and activities in cancer research throughout the world. This would provide a more suitable background than is now available for assessment of needs for support in various areas of cancer research. Toward this end, adoption of a common system(s) of classification of research projects would be useful. Attempts are being made in this direction.

III. Program Analysis Group on Cancer of the Lung

The Surgeon General's Program Analysis Group on Cancer of the Lung was first established during the spring of 1967 with Dr. Klein as Chairman and with representatives from different institutes and bureaus of the Public Health Service. In conjunction with different consultants, the group considered various aspects of the lung cancer problem including education, research, and clinical management and formulated a series of recommended programs in each category. Details of these are included in a comprehensive report submitted to the Surgeon General in July of 1967. The report included for each program, information on the nature of the problem, a description of the program including needs and anticipated obstacles, and anticipated benefits. This report also has been submitted to each member of the Lung Cancer Task Force and the Citizen's Task Force on Cancer of the Lung to serve as a working document.

ACTIVITIES OF THE SCIENTIFIC AND TECHNICAL INFORMATION OFFICE

Five interrelated activities are conducted by the Scientific and Technical Information Office:

1) THE ANNUAL "ANALYSIS OF PROJECTS SUPPORTED BY NCI IN FY 1967":

All NCI-supported projects are related to the mission of the National Cancer Institute, although the relation may not always be obvious to a casual observer. In order clearly to relate individual projects to the goals, aims, and mission of NCI, an annual Analysis is prepared in which projects are grouped together into cancer-related categories.

During the past year, a brief 3-4 line abstract was prepared for each of the 2,145 projects supported by NCI from FY 1967 funds (including all intramural projects, contracts, and grants). Related projects were grouped into 222 cancer-related categories representing subdivisions of 5 major NCI program areas: Etiology, Therapy, Cancer-related Biology, Multidisciplinary Projects, and Training. The Analysis showed how funds were obligated in terms of the 5 major program areas, 30 general scientific areas, 222 specific research sub-areas, and in terms of each of the 2,145 individual projects which were combined to form the sub-areas.

A similar Analysis will be prepared for projects supported by NCI during FY 1968.

2) AUTOMATION OF METHODS FOR PROGRAM ANALYSIS:

During FY 1968, the Scientific and Technical Information Officer began writing programs for manipulating code numbers used for analysis of research information and for computer-assisted preparation of the FY 1968 "Analysis". Several programs for the 360/50 computer have been completed and debugged.

Abstracts of individual research projects, along with administrative identification for every project supported by NCI in FY 1968 have been entered on punched cards for processing by computer programs written in PL/I. Tables appearing in the Analysis of FY 1968 projects will be prepared by the computer, using these punched cards.

3) DEVELOPMENT OF A HIERARCHICAL DECIMAL CLASSIFICATION OF BIOMEDICAL INFORMATION:

In FY 1968 the decimal classification of biomedical information was revised and expanded with particular emphasis on categories dealing directly with cancer research. Initial efforts to automate this classification are in progress. This classification is the key to the fourth activity described below.

4) INTELLECTUAL SUPPORT OF PROJECTS SUPPORTED BY NCI: TRIAL OF AN SDI SYSTEM:

Although NCI supplies grantees with funds for equipment, facilities, personnel, and other "hardware" required for their research, little software or "intellectual support" (in terms of information services) is provided. The success of research depends, in part, on the ability of a scientist to keep abreast of developments and information related to his research. One approach to supplying such information is undergoing a one-year trial in this office.

During FY 1968, about 6% of all NCI grantees (101 scientists) received summaries of published articles related to their research. The summaries were taken from the first page of each article appearing in 12 major cancer research journals. If the article was related to research conducted by the grantee, he received a copy of the summary. (Permission to copy summaries was obtained from editors of each journal.)

A decimal classification developed in this office is used to index the type of research conducted by each grantee (based on statements made in his most recent research grant application). The same classification is used to index information contained in scientific articles appearing in the 12 journals. If the number used to index an article matched a number assigned to a scientist, then the computer program identified the match, and a summary of the article was sent to the scientist. As a result of this trial, 1,389 articles were indexed, and 6,411 summaries were mailed to participating scientists. The NCI grantees who received summaries returned an "evaluation slip" for about 85% of the summaries they received.

This experimental system for selective dissemination of information (SDI) was terminated after a one-year trial. The evaluations will be used to analyze the performance of the system and to plan future services during the coming year, and a description of the trial will be prepared for publication.

5) OTHER ACTIVITIES RELATED TO SCIENTIFIC AND TECHNICAL COMMUNICATION:

The Scientific and Technical Information Office prepares replies to requests for information about the scientific and technical communications activities of the National Cancer Institute. The office is also responsible for coordinating (on an informal basis) the scientific and technical information activities of the National Cancer Institute, and the office serves as a focal point for these activities.

ANNUAL REPORT

July 1, 1967 - June 30, 1968
Murray J. Shear, Special Advisor
OADP, NCI

Information, advice, and help on various scientific matters were given in response to requests from within the NCI and NIH, from academic and commercial organizations, and from scientists abroad. Illustrative instances follow.

Chemical Carcinogenesis Planning Committee

A report on the recommendations of the Discussion Group on "Communication" was formulated, in my capacity as Chairman of that Group, and presented to Dr. Cantarow. Contributions were made to the work and to the report, of the Committee of Chairmen of all the Groups, which was prepared for presentation to the National Advisory Cancer Council.

SATCOM

As an invited "consulting correspondent", participated in the work of a conference called by the Scientific and Technical Committee (SATCOM) of the National Academy of Sciences. Subsequent activities have been carried out by mail.

Personnel

By request, evaluations and recommendations were made to this and other scientific organizations on the qualifications of candidates, both American and foreign.

International

Consultation was given on: the planning of the Fogarty International Center which is being established at the NIH; evaluation of the role of the Council of International Organizations of Medical Sciences("CIOMS") and the nature of future U.S. participation; improving the management of the International Union Against Cancer ("UICC"); etc.

Received, and conferred with, scientists from various foreign countries. By request, arrangements were made for the schedules of others who were planning to visit here. In addition, mail from scientists in other countries maintained the liaisons established previously. Among the countries represented may be mentioned Japan, U.S.S.R., Hungary, Korea, Greece.

Particularly numerous were the contacts made by Japanese cancerologists and, next in order, from Eastern Europe. By invitation, a report was edited on a visit of American scientists to Russia under the US-USSR Health Exchange program.

Public News Media

Sound scientific information, couched in terms suitable for the lay public, was provided to news media upon request. For example, the New York Times phoned to inquire about the excitement generated by TV announcements of a cure for cancer newly developed in Japan. In consequence of this interview by telephone the story by Jane E. Brody, which was printed in the Times of January 24, 1968, was first-rate.

Overall Strategy for the NCI

This assignment to the OADP constitutes a responsibility of major importance. Unstinted participation has been given in the deliberations of the group on this assignment and, as invited, on other activities of the OADP.

Research Information Branch

Office of the Director

National Cancer Institute

Program Activities Report, July 1, 1967-June 30, 1968

Public interest in the progress of cancer research was served by interpretive reporting through the public media and special information and education projects during the year.

The appointment of a Lung Cancer Task Force as directed by the President was announced in August at the first of a new series of news briefings arranged by the Department. The NCI Director, as chairman of the group, announced that development of a less hazardous cigarette would be a prime objective of the Task Force, and answered many questions put by reporters from the printed and broadcast media. A news release prepared by the Research Information Branch (RIB) was distributed. Coverage was nationwide, with stories carried by the wire services, metropolitan dailies, radio and TV networks. The event led to subsequent press and television interviews with the Task Force chairman.

On the urgent recommendation of the National Advisory Cancer Council, the NCI Director authorized RIB to proceed with a program of public education on smoking and cancer for the purpose of disseminating scientific information on the smoking problem from the National Cancer Institute as an authoritative source. Preliminary planning of this activity was begun pending an allocation of funds from the fiscal 1969 appropriation. The Assistant to the Surgeon General for Public Information appointed a committee, with the Chief of RIB as chairman, to coordinate the Institute's efforts with those of the PHS National Clearing House for Smoking and Health and the Cancer Control Branch.

"Progress Against Cancer 1967," the second public report issued by the National Advisory Cancer Council, was prepared by RIB and distributed to a selected list totaling 350,000 copies. Distribution was enlarged by the Council to include medical and dental students this year and high school and college science teachers next year. The 1967 report was devoted largely to a review of progress in virus-cancer research. The Council selected chemotherapy as the principal subject for the 1968 report, and chemical carcinogenesis for 1969. Reports on the Institute's information activities were made at the regular meetings of the Council by John Mack Carter, chairman of the Council's Subcommittee on Public Information.

A two-part television program in color on chemotherapy research, entitled "Drugs Against Cancer," was completed by National Educational Television under NCI contract. The project was originated and carried out by RIB, with technical supervision by the Scientific Director for Chemotherapy. Telecasting of the two 30-minute shows, subtitled "The Search" and "The Battle in the Cell," was begun in February to continue into August on the

133 stations of the NET network throughout the continental United States, Hawaii and Puerto Rico. The total audience is estimated at 18,000,000. Prints of the films will be distributed as motion picture subjects subsequent to broadcasting.

Special services to the news media included consultation and arrangement of interviews for writers preparing articles on different aspects of the cancer problem for *Fortune*, *Saturday Evening Post*, *Good Housekeeping*, and *Reader's Digest*. Response was made to 322 general inquiries from the news media.

RIB staff members managed news coverage arrangements for the annual meeting of the American Association for Cancer Research, the Fifth Annual Meeting of the Society for Cryobiology, and the Fourth Cryopreservation Conference--Organs. A discussion on arrangements was conducted with members of the Program Committee for the Sixth National Cancer Conference and the Vice-President for Public Information of the American Cancer Society, co-sponsor with NCI of the quadrennial meeting scheduled for September 18-20 in Denver. Details of services to science writers covering the meeting, and exhibits to be provided by the sponsors were taken up. Information personnel from both sponsoring agencies will staff the news coverage operation.

The Chief of the Branch continued his activities as a member of the Department's Career Service Board for Public Information and chairman of the subcommittee on continuing education for middle-grade information personnel, until reorganization of the Board on February 1. He addressed a group of administrative personnel of NCI on the public information function, and discussed this topic with two NIH grants associates as a step in their training. Three NIH public information interns and a management intern completed training assignments in the Branch.

A recommendation made to the Smithsonian Institution by RIB two years ago that an exhibit on cancer research be included in the new health science hall of the Museum of History and Technology was finally accepted, and the planning of a suitable display was begun in collaboration with the curator. The exhibit, which will deal with the history of cancer research, the state of the art, and future trends, is expected to be viewed by 5,000,000 visitors a year.

Distribution of publications totaled nearly 1.5 million copies. Individual requests for copies of publications continued to grow, reaching an annual volume of approximately 150,000. Employment of a temporary, part-time clerk to assist with the large volume of mailings was authorized after a management study of the problem. A management study also resulted in allocation of additional space to distribution operations.

A total of 2,616 public inquiries were answered, including letters referred by the White House and members of both houses of Congress.

As in former years, the Branch participated in the preparation of materials required for the appropriation hearings. These included the opening statement for the Director, a statement of research highlights, and special reports on various program areas.

Research and Program Reports Section

Section members continued to gather the results of research on cancer and to interpret important findings for the public, the press, and Congress. Emphasis was in areas of particular current interest--virus-cancer research and chemotherapy--and also in the developing areas of chemical carcinogenesis, immunology, and Task Force implementation.

"Progress Against Cancer" Reports

Preparation of "Progress Against Cancer 1967," the second report of the National Advisory Cancer Council to the public, was a major project. The 65-page illustrated booklet, produced by the Research Information Branch for the Council, was presented in two main parts: "Research on Prevention and Control," and "Research on Viruses and Cancer." The latter consisted of a comprehensive review of virus-cancer research from its beginnings up through recent developments. Release of the publication received wide press coverage and many requests were received from the media for extra copies and additional information.

"Progress Against Cancer 1968," highlighting research on drug treatment, is now being prepared by the Section Head.

Assistance to the Press

Non-technical summaries of papers presented at a medicinal chemistry symposium held in September during the American Chemical Society's annual meeting were prepared. The summaries, released by ACS, featured points covered by several NCI and non-government scientists in discussing the chemical and biological correlates in carcinogenesis and mutagenesis.

At the request of Public Health Reports, four non-technical summaries of papers presented by NCI staff members at the October meeting of the American Public Health Association were prepared.

A news release on the use by the Communicable Disease Center of NCI's new mobile laboratory was expanded by the Public Health Service into a larger story of research at CDC on green monkey kidney disease.

The Section prepared the announcement of the appointment the Lung Cancer Task Force made by Dr. Endicott at the news conference in August.

An interview arranged for a New York Times writer resulted in publication of a story on projections of cancer rates and risks for the United States. This generated interest in the subject on the part of a number of other science writers who received assistance in preparing their own stories.

The Section assisted Fortune magazine in preparing a story on smoking and health, with emphasis on the development of a less hazardous cigarette; and a story on cancer research in the areas of immunology, etiology, and viral-leukemia studies.

Guidance was given to the CBS program, "21st Century," in planning scenes for a telecast dealing with viral causation of cancer in man and treatment of human cancer.

Appointments with scientists were arranged for Health Bulletin, Journal of the American Medical Association and Reader's Digest, among others.

For the ninth successive year, Section members assumed responsibility for news coverage arrangements for the annual meeting of the American Association for Cancer Research held in Atlantic City April 10-13. An announcement of the meeting and news summaries of two NCI papers were prepared.

Publications

Large-scale revision and expansion of several publications for the general public were prepared during the year. The publications were "Progress Against Leukemia Research Report," "Viruses and Cancer Research Report," "Leukemias, Lymphomas and Multiple Myeloma," which replaces the site pamphlet "Leukemia;" "Cancer of the Breast," "Cancer of the Bone," and "Cancer of the Lung." A new pamphlet, "Cancer of the Bladder," was completed and a revised pamphlet on "Quackery" is being prepared.

Other Activities

Reports prepared by Section members for the Appropriations Hearings for fiscal year 1968 were "Highlights of Research during 1967," "Breast Cancer Task Force," and contributions to the "Programmed Activities Report," including sections on the Virus Program, Chemical Carcinogenesis Program, Immunology Research, Breast Cancer Task Force, and Research Instrumentation.

Approximately 30 accounts of research findings and program developments were prepared for the Director's weekly report to the Director, NIH.

An article on the surgical laser was provided for a new edition of World Book Encyclopedia.

At the request of the Clinical Center, examples of outstanding developments related to clinical research on choriocarcinoma and acute leukemia were prepared for use by Today's Health.

A Section member gave talks to 7th graders at Western Junior High School using the Science Teaching Aids developed by the Branch in cooperation with the National Science Teachers Association.

A promotional flyer for the Journal of the National Cancer Institute was prepared.

Each month more than 350 scientific journals were scanned for reports on Institute-directed or supported research. An average of 76 references per week were selected and filed for use by Branch writers and other members of the Institute staff.

Many new additions were made to the Institute archives, files consulted frequently by NCI, NIH, and PHS personnel.

Information and Education Section

The Section, in keeping with its mission of adapting information on progress in cancer research to the needs of the public, carried out its responsibilities in various ways during the year. Staff members answered inquiries from the public; prepared news releases, articles, and publications; provided information to news and feature writers on request; and arranged schedules of official visitors to the Institute. Among the Section's special responsibilities was coverage of chemotherapy research and extramural activities.

Public Inquiries

The Section answered 2,616 letter and telephone inquiries--many from cancer patients or relatives of patients. The majority were questions about proved or unproved methods of treatment; suggestions or offers of theories, methods, or materials for detecting or curing cancer; requests from students for information or specimens for reports or laboratory projects; requests from persons other than students for references, bibliographies, or general information on cancer, or questions about the role of environmental or personal factors in the cause or prevention of cancer.

Many persons requested information on new methods of treating leukemia, after reading newspaper and magazine articles about NCI-developed methods of therapy and NCI-supported leukemia studies by other institutions. Among the new treatment methods that generated considerable interest was L-asparaginase; the Section's replies concerning this new anti-cancer material emphasized that it is still in early stages of its development.

Another area where public inquiries were heavy during the year concerned new treatment methods for Hodgkin's disease, particularly a 4-drug treatment developed by NCI scientists.

There were many requests for information about the new Lung Cancer Task Force and efforts to develop a less hazardous cigarette.

A somewhat exaggerated story on NBC Television News about the difficulty experienced by two investigators at the University of Southern

California in obtaining research funds brought many inquiries to be answered, several of them from Congressmen's offices.

The Section also received an unusual number of letters and calls about the work of a Japanese professor who was reported in some American newspapers to have found a cancer cure. Our replies pointed out that the professor had claimed cure of the disease in only one patient, and his work must still be regarded as premature.

The Section answered 112 telephone and letter inquiries from Congressional offices, often on behalf of patients or their relatives. Some constituents suggested increases in financial allocations for cancer research, others requested information on cancer treatment. A total of 110 letters was referred for reply by the White House. The majority of these urged the diversion of Federal money for cancer research from space and military programs.

A total of 4,864 publications was distributed in answer to inquiries.

News Releases, Articles, and Speeches

Writers in the Section prepared 58 press releases, press announcements, news stories for the NIH Record, press summaries, and other articles. Among the stories covered in the releases were: the laser beam as a surgical tool; the study of algae extracts as potential cancer treatment sources; the establishment of the NCI-sponsored African lymphoma center; the appointments of new members of the National Advisory Cancer Council; plants from India as a potential source of anti-cancer drugs; cell biology research at the University of Pennsylvania under an NCI grant; announcements of the retirement of Dr. Howard B. Andervont and the appointment of Dr. Jesse S. Steinfeld; announcements of the need for certain types of research patients in the Institute's clinical program; reports of new or revised publications, such as "Drugs vs. Cancer" and "Treating Cancer"; and the announcement of the NCI-sponsored National Educational Television program, "Drugs Against Cancer."

Within the Branch, the Section coordinated the production of columns for the "Research for Health" series syndicated to weekly and daily newspapers by OI-NIH. About one column a month was supplied, covering research in such fields as Hodgkin's disease, malignant melanoma, cancer statistics, and mycotoxins. The Section Head prepared two speeches for use by the Assistant Director, NCI. Eight Weekly Report items were prepared, dealing with chemotherapy and special meetings and conferences.

Publications

"Drugs vs. Cancer" (Research Report No. 3) was prepared in the Section and is being extensively used in answering public inquiries about cancer chemotherapy.

The Section also provided assistance on topics relating to cancer chemotherapy for other publications by the Branch.

Assistance to News Media

Staff of the Section, at the request of various news media representatives, was helpful in the development of numerous stories. Some examples of this assistance were: to Tom Shachtman, writer for the CBS program "21st Century," on instrumentation in cancer research; Good Housekeeping Magazine on two stories--cancer quacks; and the carcinogenicity of charcoal-broiled meats; Station WAVE, Louisville, Kentucky, for a television interview of the Director, NCI, on smoking and health; to Phil Gunby, of JAMA, on a number of news articles dealing with clinical and non-clinical research; Alice Lake, free-lancing a Saturday Evening Post story on leukemia; Pierre Bourget, French Radio/TV, on viruses and cancer; Bill Small, of Business Week, on leukemia; Gene Bylinsky, of Fortune, on two stories dealing with cancer in general, and smoking and health; and Tom O'Toole, of the Washington Post, on leukemia. Certain developments in cancer or cancer-related areas brought requests for information from many newsmen. Among these developments were: the Strickman cigarette filter sponsored by Columbia University; the three-dimensional photograph of a cancer cell published by Dr. Ambrose of the Chester Beatty Institute, London; our announcement of the laser beam as an experimental surgical tool, and the publication of "Drugs vs. Cancer" and "Progress Against Cancer 1967." The Section also arranged a mail interview of the Scientific Director for General Laboratories and Clinics, NCI, with the Science Editor of the Newark Star-Ledger. In all, the Section handled 221 press inquiries during the year.

Visitors

The Section arranged the official visits of 9 prominent persons to NCI by scheduling appointments for them, escorting them to appointments, assisting them with transportation, and providing background information. The visitors represented Togo, the Netherlands, Ghana, Iran, England, Yugoslavia, Spain, and Turkey. The representative of Togo was his country's Ambassador to the United States, a diplomat who is also a cancer surgeon. A visit was also arranged for Donald A. Weadon, Assistant to the Publisher of Life Magazine, one of whose missions is to give talks to the U.S. business community about advances in medical research and other public affairs concerns.

Allied Activities

National Educational Television. The Head of the Section and Assistant Head were Project Officer and Assistant Project Officer respectively on a contract with National Educational Television for the production of "Drugs Against Cancer," two half-hour films in color on cancer chemotherapy that were shown on 133 stations of the NET network.

Cancer Month Proclamation. The Section prepared for the President the draft of the annual proclamation for Cancer Control Month which he signed and issued before April 1.

Meeting Coverage. A Section staff member took charge of the news room at the 1968 Annual Meeting of the American Association for Cancer Research, and provided assistance to the reporters covering the gathering. The same staff member also represented the Institute in the news room at the Acute Leukemia and Burkitt's Lymphoma Conference, in Boston. The Section Head and Assistant Head, who will share responsibility for the press room with American Cancer Society information staff at the Sixth National Cancer Conference in Denver, have made the necessary preliminary arrangements for this meeting.

Special Reports. Section staff members prepared two special reports that were submitted in conjunction with the Appropriations Hearings of the House and Senate for fiscal year 1969. The subjects were acute leukemia, and public information programs.

Special Mailing. As a result of press coverage following the combined clinical staff meeting on Hodgkin's disease in the Clinical Center Auditorium in February 1967, the Section responded to appeals from 70 professional persons (physicians, nurses, hospital staff people) for detailed information by sending them reprints of the proceedings of this meeting, along with other pertinent material on the disease.

Cancer Bulletin Article. The Section Head prepared an article on NCI history and programs to be included in a special issue of the Cancer Bulletin published by the University of Texas M.D. Anderson Hospital and Tumor Institute. Articles for the entire issue were written by NCI staff.

Publications, Visuals, and Reference Section

This Section planned, organized, and carried out projects designed to produce and disseminate various types of printed and audiovisual materials on cancer. The continuing increase in the number of requests for materials on cancer by both the general and professional publics as well as students and teachers from all levels of learning is indicative of the ever-increasing awareness of the cancer problem.

Other activities of the Section included assisting with the planning of Branch projects, maintenance of a reference library, handling clearance of nonresearch materials, and screening and circulation of journals for the Branch.

Because of the increase in workload, space and management surveys were made resulting in allocation of additional space and a part-time clerk-typist position.

Publications and Audiovisuals

Science teaching aids. The project of preparing and distributing teaching aids to science teachers and supervisors was completed during the year. Two filmstrips with sound tapes, "What Science Knows About Cancer," and "Aberrant Cells - The Nature of Cancer," were completed and distributed to 25,000 science teachers who requested them. Some 475,000 copies of other teaching aids, including a portfolio of photographs for classroom use, were distributed. Requests for these materials came from teachers in training, and science, health and physical education teachers. Although the special project has been completed, requests for the materials continue. The filmstrips are now for sale by the producer, and reprintings of the other materials are being made at the Government Printing Office.

Progress Against Cancer 1967. The second report by the National Advisory Cancer Council was published and 350,000 copies were distributed to physicians, medical and dental students, Members of Congress, health officers, and others interested in the subject. The Section worked out illustrations, layout, printing and distribution details.

Other Publications and Audiovisual Materials. Nearly 1½ million publications were distributed this year, an increase over last year. Publications were distributed by planned patterns of new and revised publications, response to inquiries, and at exhibits. Twenty releases were sent to the news media.

The publication, "Research and Related Programs of the National Cancer Institute," was brought up-to-date by the Section for reprinting.

Printing and illustrations for all publications produced by the Branch were handled by the Section, and assistance was given to other NCI areas in obtaining printing of scientific publications.

A new exhibit, "Cancer Research Centers," was prepared for the Regional Medical Programs Conference. This exhibit and existing ones were shown throughout the year upon request, being refurbished and brought up-to-date as necessary. Several have been on long-term display at the Medical Museum of the AFIP and Smithsonian Institution where they are seen by millions of persons.

The Section is again preparing exhibits for the Sixth National Cancer Conference, September 18-20, 1968, a meeting sponsored by the American Cancer Society and the Institute every 4 years. These exhibits will include a display of medical books to be supplied by book publishers; a publications and journals exhibit, incorporating ACS and NCI publications and journals as well as other leading cancer journals; and an NCI exhibit, "The Search for Cancer Drugs," being prepared in cooperation with the CCNSC.

Photographs, charts, and slides were prepared on a continuing basis. Assistance was provided to the news media in making photographs and television films.

Reference Service

Some 2,000 cancer and closely related articles were clipped from 7 newspapers to which the Branch subscribes. These articles are circulated to the Branch staff and retained for reference use. Cancer articles were marked in 45 journals and other printed materials on cancer and were circulated to the staff.

These materials and other reference items are retained in the reference library for use by the Institute staff.

Clearance of Nonresearch Materials

Over 125 nonresearch items prepared by the NCI staff for publication or other official use were received for clearance.

Monthly reports of the material cleared and in clearance were prepared and submitted to the Board of Editors to be appended to its report of research materials received for clearance.

Assistance to Scientific Groups

The Section Head, by request, provided pre-meeting assistance to the Press Committee of the Society for Cryobiology for its fifth annual meeting and to the Cryopreservation Conference on Organs. News room assistance was given to the Society last August during their fourth annual meeting.

NATIONAL CANCER INSTITUTE

ANNUAL REPORT

CHEMOTHERAPY

July 1, 1967 through June 30, 1968

NATIONAL CANCER INSTITUTE
CHEMOTHERAPY

Annual Report of the Scientific Director
July 1, 1967 - June 30, 1968

The annual reports of 1966 and 1967 described efforts to guide the Chemotherapy program toward the specific goals of drug control of human cancer defined in the extensive appraisal of 1965(1). The work of the past two years has effected a major refinement of goals for the drug control of fast growing and slow growing tumors, leading in turn to changes in the drug development program, clinical trials and laboratory research.

The major development is the growing recognition that rapidly growing tumors are more drug sensitive than slow growing tumors. The explanation for this difference is obscure. The most attractive working hypothesis is that rapidly growing tumors have a much larger fraction of their cells in the mitotic (or proliferative) cycle and are therefore at risk to agents that kill cells during DNA synthesis or other phases of the cycle (cell cycle specific drugs). Presumably, the great majority of the cells of the slow growing tumors are not in the proliferative pool (not in cycle) and not at risk from cell cycle specific drugs. Finally the toxicity of cell cycle specific drugs for normal cells is most marked in rapidly growing tissues such as bone marrow or gastrointestinal epithelium. Despite this, it is quite heartening to recognize that there are sufficient differences between the proliferative pools of the fast growing tumors and of normal fast growing cells, that the drugs can achieve great selectivity in killing tumor cells. This exploitation of selective toxicity depends upon the construction of a dosage schedule that will expose a maximal number of tumor cells to tumoricidal drug concentrations during their mitotic cycle, and a minimal number of normal cells during the same period. It is clear that optimal use of the selective tumor killing capacity of cell cycle specific drugs, depends upon a custom tailoring of dosage schedule to the specifically sensitive phases of mitotic cycles of the tumor and host normal cells.

The import of these concepts for the drug development program is substantial. During the past year the senior staff of chemotherapy has redesigned the entire program in light of the new data. In the elaboration of program logic the staff received extensive advice from our advisory groups and individual scientists. In addition Mr. Louis Carrese and the staff of the NCI planning office gave assistance in detailing the decision networks and in over all systems analysis. The present program contains major changes over that established in 1965(1). With specific objectives of drug control of cancer as they are emerging it is possible to concentrate the planning of drug development. Screening, toxicology and pharmacologic disposition have been shifted so as to point as precisely as possible in the direction of the targets now seen: the interdependence of growth rates, drug susceptibility and dosage scheduling. This sharpened purpose also brings into focus program gaps, and highlights the specific research that will fill the need. For example, there are excellent systems for developing drugs to control fast growing tumors yet these have failed to find agents for the slow growers.

Either we need different systems to find fundamentally different drugs for the slow growing tumors or we need to use present drugs in a very much different way to match the unique biology of the slow growers. It is possible to specify with considerable accuracy the kinds of data needed.

Clinical trials require change. One frustration of clinical trials is the bewildering variety of cancers in which therapeutic effectiveness can be studied. How many papers has each of us read in which a new drug has been tried in 50 patients - 10 patients with lung cancer, and 6 patients with breast cancer and 1 or 2 patients each with different other tumors? With the clear break between success in the drug control of fast growers and failure in the slow growers, it seems reasonable in the redefined program to choose as initial therapeutic targets a few examples of each, but to be certain that at least 20 patients with each histologic type will be treated. The fast growing tumors chosen are the acute leukemias and reticulum cell sarcoma; the slow growers - colon, breast, and lung cancer. Melanomas have been added as intermediate in growth rate.

A second problem has arisen about clinical trials. The present need in the clinic, as the penultimate step in the drug development program, is for highly individualized study of single patients, trying to tie together growth rate, size of proliferative pool, drug susceptibility and dosage schedule delivering drug to the target site in a way that will kill tumor cells and spare host cells. Such studies require the assembling of specialized teams of physicians and scientists who can concurrently and validly make the needed assessments. This requires the expansion of direct operations both in our own clinics and in contracts. The difficulty appears in that the present major organizational structure for clinical trials lies within the cooperative groups. While a few of the groups are in a position to carry out this step in drug development, for the most part their activities revolve about a much later step in cancer chemotherapy - the comparison of therapies and the incorporation of new but proven active agents into medical practice. In addition the cooperative groups provide a stable funding base for free-ranging excursions into clinical investigation of cancer, for the development of improved total care of the advanced cancer patient, for rapid communications and for the education of students and physicians. While there is a clear need for the Chemotherapy program to elaborate its already large communications and service networks for the cooperative groups, it is equally clear that the key early steps in the drug development program must be carried out much more directly.

There are two major aspects of the Chemotherapy program of NCI - research and development. The latter is the drug development program. The research aspect is at least of equal importance if the Chemotherapy program is to continue to evolve and to improve. Research in the Chemotherapy program is not an added afterthought but is present in every component. It is a major part of direct activities in our laboratories and clinics at Bethesda and Baltimore; it is an intimate though smaller part of the work of our major contractors; it is present in the laboratories and clinics of a large number of grantees who work closely with our program. While part of the research is completely free-ranging, most of it has its inspiration in the unanswered problems generated by the drug control of tumors. What are the biological and molecular differences between slow growing and fast growing tumors; how can these be measured; what happens to cells when they cease mitotic activity yet retain the ability to return to the proliferative pool; how can we control the movement of tumor cells from a drug protected phase into a drug sensitive phase ---? Fortunately these and many other fundamental questions on cell biology are of intense interest to our scientists and physicians both those in the program and those closely allied to it. From the answers they are providing will come an understandable, and hopefully complete, drug control of cancer.

1. Zubrod, C. G., Schepartz, S., Leiter, J., Endicott, K. M., Carrese, L. M., and Baker, C. G.: The Chemotherapy Program of the NCI: History, Analysis, and Plans, Cancer Chemother. Rep., Vol. 50, No. 7, October 1966.

TABLE I

ANALYSIS OF CONTRACT ACTIVITIES IN THE CHEMOTHERAPY PROGRAM

TYPE OF ACTIVITY	NO. OF CONTRACTS		ANNUAL LEVEL[1]		(Percent)
TOTAL	146[2]		$21,713,000		
I. DRUG PROCUREMENT			3,765,000		(17.3)
A. Preparative Laboratories					
1. Clinical	7	$ 810,000[3]		(3.7)	
2. Non-Clinical	7	570,000[3]		(2.6)	
B. New Synthesis	10	1,600,000[3]		(7.4)	
C. Quality Control, Dosage Formulation, etc.	7	785,000[3]		(3.6)	
II. NATURAL PRODUCTS DEVELOPMENT			2,610,000		(12.0)
A. Antibiotic Fermentation & Isolation	4	495,000		(2.3)	
B. Plant and Other Natural Product Collection and Isolation	13	814,000		(3.7)	
C. Related Assay Testing	11	806,000		(3.7)	
D. Production for Pharmacology & Clinic	5	495,000		(2.3)	
III. DRUG EVALUATION			4,188,000		(19.3)
A. Primary *In Vivo* Screening	8	1,184,000		(5.5)	
B. *In Vitro* Screening	5	142,000		(.6)	
C. Detailed Evaluation of New & Old Drugs	9	1,473,000		(6.8)	
D. Toxicology and Pharmacology	10	1,389,000		(6.4)	
IV. CLINICAL TRIALS			3,476,000		(16.1)
A. Patient Supportive Care	5	581,000		(2.7)	
B. Clinical Experimental Therapeutics	8	2,490,000		(11.5)	
C. Miscellaneous	9	405,000		(1.9)	
V. ANIMAL PROCUREMENT & SUPPORTING SERVICES			3,539,000		(16.3)
A. Animal Production	41	2,576,000[3]		(11.8)	
B. Disease Control	4	251,000[3]		(1.2)	
C. Trust Stocks & Genetic Control	11	614,000		(2.8)	
D. Tumor & Cell Culture Banks	3	98,000		(.5)	
VI. RESEARCH SERVICES			749,000		(3.4)
A. Data Processing	6	545,000		(2.5)	
B. Statistical Services (Clinical)	1	9,000		(-.-)	
C. Literature, Publications and Related Activities	5	123,000		(.6)	
D. Miscellaneous	4	72,000		(.3)	
VII. RESEARCH AND DEVELOPMENT			3,386,000		(15.6)
A. New Screening Methods	13	1,304,000		(6.0)	
B. Biochemical Mechanisms of Action and Resistance	6	449,000		(2.1)	
C. Pharmacology & Physiological Disposition	7	1,463,000		(6.7)	
D. Immunity & Immune Response	1	170,000		(.8)	

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[1] Funds obligated during Fiscal Year 1968. Not included are funds utilized for direct purchase of clinical drugs (approximately $550,000).

[2] Since many contracts involve more than one activity, the totals of I - VII do not correspond with the total number of contracts.

[3] Not included in this amount is the cost of one or more on-going contracts in which no Fiscal Year 1968 funds were obligated. See Table II for individual contracts involved.

TABLE II
ANALYSIS OF CONTRACTS
BY ACTIVITY

	ACTIVITY CONTRACT	DOLLAR LEVEL[1]		
I.	DRUG PROCUREMENT			$ 3,765,000
A.	Preparative Laboratories			
1.	Clinical		$ 810,000	
	Aerojet General Corp. (68-)	$ 138,000		
	Collaborative Research (66-506)	132,000		
	Merck and Company (62-479)	226,000		
	Monsanto Research Corp. (65-1044)	110,000		
	Monsanto Research Corp. (67-658)	125,000		
	Parke Davis & Company (4320)	(32,000)[2]		
	Starks Associates (62-484)	79,000		
2.	Non-Clinical		570,000	
	Merck and Company (62-479)	200,000		
	Monsanto Research Corp. (65-1044)	16,000		
	Monsanto Research Corp. (67-658)	20,000		
	Parke Davis & Company (4320)	(60,000)[2]		
	Stanford Research Inst. (68-)	172,000		
	Starks Associates (62-484)	90,000		
	Starks Associates (68-)	72,000		
B.	New Synthesis		1,600,000	
	Connecticut, Univ. of (65-1035)	11,000		
	Georgia Inst. Technology (68-)	30,000		
	Little, Arthur D., Inc. (66-502)	54,000		
	Michigan, University of (68-)	48,000		
	Microbiological Assoc. (65-587)	215,000		
	Midwest Research Inst. (65-94)	321,000		
	Pfizer, Chas., & Co. (68-45)	25,000		
	Southern Research Inst. (64-51)	388,000		
	Stanford Research Inst. (64-500)	380,000		
	Utah, University of (65-1041)	128,000		
C.	Quality Control, Dosage Formulation, etc.		785,000	
	Ben Venue Labs., Inc. (66-909)	100,000		
	Burrough Brothers (65-2)	53,000		
	Michigan, University of (68-)	69,000		
	Microbiological Assoc. (65-587)	135,000		
	Pfizer, Chas., & Co. (67-687)	250,000		
	Pittsburgh, Univ. of (67-1186)	(22,000)[2]		
	Stanford Research Inst. (64-500)	178,000		

TABLE II (Continued)

	ACTIVITY CONTRACT	DOLLAR LEVEL[1]		
II.	NATURAL PRODUCTS DEVELOPMENT			$ 2,610,000
A.	Antibiotic Fermentation & Isolation		$ 495,000	
	Bristol Laboratories (64-1159)	$ 54,000		
	Pfizer, Chas., & Co. (68-45)	180,000		
	Squibb, E. R., & Sons (65-1056)	103,000		
	Upjohn Company (62-168)	158,000		
B.	Plant & Other Natural Product Collection & Isolation		814,000	
	Agriculture, Dept. of (64-1)	120,000		
	Arizona, Univ. of (67-1484)	95,000		
	Butterfly Art Jewelry (68-893)	8,000		
	Commonwealth Sci. & Indus. (64-522)	(29,000)[2]		
	Costa Rica, Univ. of (64-31)	12,000		
	Hawaii, Univ. of (67-748)	22,000		
	Miami, Univ. of (67-1179)	29,000		
	Nuclear Services Corp. (68-)	30,000		
	Pfizer, Chas., & Co. (68-45)	203,000		
	Research Triangle Inst. (4322)	123,000		
	Tyco Laboratories, Inc. (67-723)	32,000		
	Wisconsin Alum. Res. Fdn. (67-747)	50,000		
	Wisconsin, Univ. of (64-551)	90,000		
C.	Related Assay Testing		806,000	
	Battelle Memorial Inst. (62-495)	95,000		
	Bristol Laboratories (64-1159)	85,000		
	Hazleton Laboratories (63-622)	86,000		
	I.I.T. Research Inst. (4349)	61,000		
	Little, Arthur D., Inc. (65-61)	40,000		
	Michigan Dept. Health (65-7)	32,000		
	Pfizer, Chas., & Co. (68-45)	225,000		
	Southern Research Inst. (65-594)	50,000		
	Upjohn Company (62-168)	12,000		
	Wisconsin Alum. Res. Fdn. (66-494)	100,000		
	Wisconsin, University of (67-1126)	20,000		
D.	Production for Pharmacology and Clinic		495,000	
	Bristol Laboratories (64-1159)	3,000		
	Commonwealth Sci. & Indus. (64-522)	15,000		
	Pfizer, Chas., & Co. (68-45)	125,000		
	Squibb, E. R., & Sons (65-1056)	300,000		
	Upjohn Company (62-168)	52,000		

TABLE II (Continued)

ACTIVITY CONTRACT	DOLLAR LEVEL[1]		
III. DRUG EVALUATION			$ 4,188,000
A. Primary In Vivo Screening		$1,184,000	
Battelle Memorial Inst. (62-495)	$ 120,000		
Bristol Laboratories (64-1159)	14,000		
Hazleton Laboratories (63-622)	210,000		
I.I.T. Research Institute (4349)	175,000		
Little, Arthur D., Inc. (65-61)	120,000		
Microbiological Assoc. (64-911)	55,000		
Southern Research Inst. (65-594)	350,000		
Wisconsin Alum. Res. Fdn. (66-494)	140,000		
B. In Vitro Screening		142,000	
Bristol Laboratories (64-1159)	2,000		
Little, Arthur D., Inc. (65-61)	20,000		
Miami, University of (64-890)	52,000		
Upjohn Company (62-168)	68,000		
Yale Univ. School Med. (67-1193)	(13,000)[2]		
C. Detailed Evaluation of New & Old Drugs		1,473,000	
Battelle Memorial Inst. (62-495)	52,000		
Bristol Laboratories (64-1159)	5,000		
Hazleton Laboratories (63-622)	70,000		
I.I.T. Research Institute (4349)	28,000		
Little, Arthur D., Inc. (65-61)	173,000		
Microbiological Assoc. (64-911)	370,000		
Pfizer, Chas., & Co. (68-45)	65,000		
Southern Research Inst. (65-594)	665,000		
Wisconsin Alum. Res. Fdn. (66-494)	45,000		
D. Toxicology and Pharmacology		1,389,000	
California, Univ. of (68-)	188,000		
Hazleton Laboratories (63-1168)	215,000		
I.I.T. Research Institute (67-1141)	85,000		
Little, Arthur D., Inc. (65-61)	217,000		
Mason Research Institute (65-51)	199,000		
Microbiological Assoc. (64-911)	120,000		
Pfizer, Chas., & Co. (68-45)	165,000		
Pico Laboratories (67-1167)	35,000		
South Shore Lab., Inc. (64-932)	165,000		
Stanford Research Inst. (67-1143)	(80,000)[2]		

TABLE II (Continued)

ACTIVITY CONTRACT		DOLLAR LEVEL[1]	
IV. CLINICAL TRIALS			$ 3,476,000
A. Patient Supportive Care		$ 581,000	
American Natl. Red Cross (64-102)	$ 60,000		
Children's Cancer Res. (66-1147)	92,000		
Children's Hosp. Phila. (66-1152)	105,000		
I.B.M. Corporation (64-100)	320,000		
I.B.M. Corporation (68-968)	4,000		
B. Clinical Experimental Therapeutics		2,490,000	
Kentucky, Univ. of (67-1173)	(14,000)[2]		
Makerere University (67-1343)	50,000		
New York University (67-1174)	11,000		
Ohio State Univ. (67-1175)	(13,000)[2]		
Public Health Service (64-3)	1,381,000		
Veterans Adm. (64-4)	1,000,000		
Wake Forest Univ. (68-950)	6,000		
Walter Reed Gen. Hosp. (64-5)	42,000		
C. Miscellaneous		405,000	
American Cancer Society (68-51)	3,000		
Applied Radiation Corp. (67-725)	7,000		
California, Univ. of (68-)	21,000		
Chicago, University of (67-1323)	(29,000)[2]		
Hazleton Laboratories (68-)	81,000		
Kaman Nuclear Corp. (68-)	31,000		
Litton Industries, Inc. (68-)	199,000		
Perkin-Elmer Corp. (66-1159)	12,000		
Pfizer, Chas., & Co. (68-48)	51,000		
V. ANIMAL PROCUREMENT AND SUPPORTING SERVICES			$ 3,539,000
A. Animal Production		2,576,000	
Battelle Memorial Inst. (62-488)	60,000		
Bellaire Acres (68-683)	69,000		
Berkeley Pacific Labs. (68-22)	44,000		
Chas. River Breed. Labs. (68-20)	92,000		
Chas. River Breed. Labs. (68-87)	45,000		
Dublin Lab. Animals (68-23)	54,000		
Dublin Lab. Animals (68-680)	63,000		
Engle's Hamstery (68-28)	19,000		
Harlan Industries, Inc. (68-957)	40,000		
Jackson Laboratory (68-35)	249,000		
Kansas, University of (64-64)	48,000		
Laboratory Supply Co. (68-1)	20,000		

TABLE II (Continued)

ACTIVITY CONTRACT	DOLLAR LEVEL[1]	
V. ANIMAL PROCUREMENT AND SUPPORTING SERVICES (Continued)		
A. Animal Production (Continued)		
Laboratory Supply Co. (68-2)	$ 33,000	
Laboratory Supply Co. (68-19)	67,000	
Laboratory Supply Co. (68-681)	70,000	
Laboratory Supply Co. (68-956)	51,000	
Lakeview Hamster Colony (68-26 & 68-638)	24,000	
Marshall Res. Animals (68-39)	16,000	
Microbiological Assoc. (62-161)	320,000	
Millerton Research Farm (68-691)	28,000	
Pfizer, Chas., & Co. (68-45)	95,000	
Rawley Farms (68-625 & 68-703)	123,000	
Roma Kennels (68-3)	9,000	
Schmidt, A. R., Co. (66-484)	65,000	
Schmidt, A. R., Co. (67-110)	98,000	
Schmidt, A. R., Co. (68-21)	88,000	
Schmidt, A. R., Co. (68-682)	66,000	
Simonsen Labs., Inc. (65-55)	48,000	
Simonsen Labs., Inc. (68-32)	20,000	
Simonsen Labs., Inc. (68-698)	87,000	
Southern Animal Farms (68-31)	35,000	
Southern Animal Farms (68-701)	51,000	
Taconic Farms, Inc. (68-695)	65,000	
Texas Inbred Mice Co. (68-88)	172,000	
Texas Inbred Mice Co. (68-696)	39,000	
Texas Inbred Mice Co. (68-962)	40,000	
Vinemont Animal Farms (68-655)	28,000	
Zucca's Hamstery (68-27)	18,000	
Zucca's Hamstery (68-637)	17,000	
B. Disease Control		$ 251,000
Battelle Memorial Inst. (62-488)	45,000	
I.I.T. Research Inst. (4349)	80,000	
Natl. Acad. Sci. (64-44)(T.O. #6)	15,000	
New Jersey Coll. Med. (66-52)	111,000	
C. Trust Stocks and Genetic Control		614,000
Battelle Memorial Inst. (62-488)	75,000	
Chas. River Breed Labs. (66-87)	230,000	
Health Research, Inc. (65-44)	50,000	
Kansas, University of (64-44)	18,000	
Schmidt, A. R., Co. (66-86)	78,000	
Schmidt, A. R., Co. (66-484)	30,000	
Schmidt, A. R., Co. (66-901)	39,000	
Schmidt, A. R., Co. (67-110)	32,000	

TABLE II (Continued)

ACTIVITY CONTRACT		DOLLAR LEVEL[1]	
V. ANIMAL PROCUREMENT AND SUPPORTING SERVICES (Continued)			
C. Trust Stocks and Genetic Control (Continued)			
Simonsen Labs., Inc. (65-55)	$ 14,000		
Texas Inbred Mice Co. (68-88)	48,000		
Tulane University (65-593)	(83,000)[2]		
D. Tumor and Cell Culture Banks		$ 98,000	
Little, Arthur D., Inc. (65-61)	43,000		
Microbiological Assoc. (64-911)	25,000		
Southern Research Inst. (65-594)	30,000		
VI. RESEARCH SERVICES			$ 749,000
A. Data Processing		545,000	
Chemical Abstracts Serv. (65-1021)	20,000		
Control Data Corporation (67-1356)	52,000		
Cornell Univ. Med. Coll. (67-1337)	(94,000)[2]		
Leasco Sys. & Res. Corp. (68-30)	423,000		
Little, Arthur D., Inc. (65-61)	50,000		
New York University (67-1336)	(60,000)[2]		
B. Statistical Services (Clinical)		9,000	
Michigan, University of (65-45)	9,000		
C. Literature, Publications and Related Activities		123,000	
Geo. Washington Univ. (67-1338)	40,000		
Japanese Cancer Assoc. (66-77)	5,000		
Leasco Sys. & Res. Corp. (68-30)	60,000		
Medical Literature, Inc. (67-1353)	4,000		
Natl. Bureau of Standards (68-45)	14,000		
D. Miscellaneous		72,000	
Natl. Acad. Sci. (64-44)(T.O. #16)	18,000		
Natl. Acad. Sci. (64-44)(T.O. #19)	11,000		
Natl. Acad. Sci. (64-44)(T.O. #41)	13,000		
Natl. Educational TV (67-1196)	30,000		

TABLE II (Continued)

ACTIVITY CONTRACT		DOLLAR LEVEL[1]	
VII. RESEARCH AND DEVELOPMENT			$ 3,386,000
A. New Screening Methods		$1,304,000	
Battelle Memorial Inst. (62-495)	$ 9,000		
Hazleton Laboratories (63-622)	4,000		
Hazleton Laboratories (66-84)	385,000		
I.I.T. Research Inst. (4349)	8,000		
Little, Arthur D., Inc. (65-61)	296,000		
Miami, University of (64-890)	5,000		
Microbiological Assoc. (64-911)	190,000		
Pfizer, Chas., & Co. (68-45)	52,000		
Southern Research Inst. (63-68)	5,000		
Southern Research Inst. (65-594)	275,000		
Southern Research Inst. (66-29)	50,000		
Upjohn Company (62-168)	18,000		
Wisconsin Alum. Res. Fdn. (66-494)	7,000		
B. Biochemical Mechanisms of Action and Resistance		449,000	
Children's Cancer Res. (66-541)	10,000		
Scripps Clinic & Res. Fdn. (65-14)	54,000		
Southern Research Inst. (65-594)	30,000		
Southern Research Inst. (66-29)	174,000		
Stanford Research Inst. (65-575)	116,000		
Upjohn Company (62-168)	65,000		
C. Pharmacology and Physiological Disposition		1,463,000	
Children's Cancer Res. (66-541)	42,000		
Little, Arthur D., Inc. (65-61)	491,000		
Microbiological Assoc. (64-911)	250,000		
Pfizer, Chas., & Co. (68-45)	114,000		
Southern Research Inst. (64-654)	222,000		
Texas, University of (66-1156)	200,000		
Upjohn Company (62-168)	144,000		
D. Immunity and Immune Response		170,000	
Microbiological Assoc. (64-911)	170,000		

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[1] Funds obligated during Fiscal Year 1968. Not included are funds utilized for direct purchase of clinical drugs (approximately $550,000).

[2] Not included in dollar totals since no Fiscal Year 1968 funds were obligated; included here for reference since it is an on-going contract.

TABLE III
ANALYSIS OF ACTIVITIES
BY CONTRACT

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Aerojet-General Corporation (68-)			
IA1	Clinical Preparative Laboratories		$ 138,000
Agriculature, Department of (Transfer - 64-1)			
IIB	Plant & Other Natural Prod. Coll. & Isolation		120,000
American Cancer Society (68-51)			
IVC	Clinical Trials (Miscellaneous)		3,000
American National Red Cross (64-102)			
IVA	Patient Supportive Care		60,000
Applied Radiation Corporation (67-725)			
IVC	Clinical Trials (Miscellaneous)		7,000
Arizona, University of (67-1484)			
IIB	Plant & Other Natural Prod. Coll. & Isolation		95,000
Battelle Memorial Institute (62-488)			135,000
VA	Animal Production	60,000	
VC	Trust Stocks & Genetic Control	75,000	
Battelle Memorial Institute (62-495)			276,000
IIC	Natural Product Assay	95,000	
IIIA	Primary *In Vivo* Screening	120,000	
IIIC	Detailed Evaluation of New & Old Drugs	52,000	
VIIA	Screening R&D	9,000	
Battelle Memorial Institute (63-18)			
VB	Animal Disease Control		45,000
Bellaire Acres (68-683)			
VA	Animal Production		69,000
Ben Venue Laboratories, Inc. (66-909)			
IC	Quality Control, Dosage Form, etc.		100,000
Berkeley-Pacific Laboratories (68-22)			
VA	Animal Production		44,000

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Bristol Laboratories (64-1159)			$ 163,000
IIA	Antibiotic Fermentation & Isolation	54,000	
IIC	Natural Product Assay	85,000	
IID	Production for Pharmacology & Clinic	3,000	
IIIA	Primary *In Vivo* Screening	14,000	
IIIB	*In Vitro* Screening	2,000	
IIIC	Detailed Evaluation of New and Old Drugs	5,000	
Burrough Brothers Pharmaceuticals, Inc. (65-2)			
IC	Quality Control, Dosage Form., etc.		53,000
Butterfly Art Jewelry, Inc. (68-983)			
IIB	Plant & Other Natural Prod. Coll. & Isolation		8,000
California, University of (68-)			
IIID	Toxicology & Pharmacology		188,000
California, University of (68-)			
IVC	Clinical Trials (Miscellaneous)		21,000
Charles River Breeding Laboratories, Inc. (66-87)			
VC	Trust Stocks & Genetic Control		230,000
Charles River Breeding Laboratories, Inc. (68-20)			
VA	Animal Production		92,000
Charles River Breeding Laboratories, Inc. (68-87)			
VA	Animal Production		45,000
Chemical Abstracts Service (65-1021)			
VIA	Data Processing		20,000
Chicago, University of (67-1323)			
IVC	Clinical Trials (Miscellaneous)		(29,000)[1/]
Children's Cancer Research Foundation (66-541)			52,000
VIIB	Biochemical Mechanisms	10,000	
VIIC	Pharmacology & Physiological Disposition	42,000	
Children's Cancer Research Foundation (66-1147)			
IVA	Patient Supportive Care		92,000
Children's Hospital of Philadelphia (66-1152)			
IVA	Patient Supportive Care		105,000

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Collaborative Research, Inc. (66-506)			
	IA1 Clinical Preparative Laboratories		$ 132,000
Commonwealth Scientific & Industrial Research Organization (64-522)			15,000
	IIB Plant & Other Natural Prod. Coll. & Isolation	(29,000)1/	
	IID Production for Pharmacology & Clinic	15,000	
Connecticut, University of (65-1035)			
	IB New Synthesis		11,000
Control Data Corporation (67-1356)			
	VIA Data Processing		52,000
Cornell University Medical College (67-1337)			
	VIA Data Processing		(94,000)1/
Costa Rica, University of (64-31)			
	IIB Plant & Other Natural Prod. Coll. & Isolation		12,000
Dublin Laboratory Animals (68-23)			
	VA Animal Production		54,000
Dublin Laboratory Animals (68-680)			
	VA Animal Production		63,000
Engle's Hamstery (68-28)			
	VA Animal Production		19,000
George Washington University (67-1338)			
	VIC Literature, Publications & Related Activities		40,000
Georgia Institute of Technology (68-)			
	IB New Synthesis		30,000
Harlan Industries, Inc. (68-957)			
	VA Animal Production		40,000
Hawaii, University (67-748)			
	IIB Plant & Other Natural Prod. Coll. & Isolation		22,000

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Hazleton Laboratories, Inc. (63-622)			$ 370,000
IIC	Natural Product Assay	86,000	
IIIA	Primary *In Vivo* Screening	210,000	
IIIC	Detailed Evaluation of New & Old Drugs	70,000	
VIIA	Screening R&D	4,000	
Hazleton Laboratories, Inc. (63-1168)			
IIID	Toxicology & Pharmacology		215,000
Hazleton Laboratories, Inc. (66-84)			
VIIA	Screening R&D		385,000
Hazleton Laboratories, Inc. (68-)			
IVC	Clinical Trials (Miscellaneous)		81,000
Health Research, Inc. (65-44)			
VC	Trust Stocks & Genetic Control		50,000
Illinois Institute of Technology Research Institute (4349)			352,000
IIC	Natural Product Assay	61,000	
IIIA	Primary *In Vivo* Screening	175,000	
IIIC	Detailed Evaluation of New & Old Drugs	28,000	
VB	Animal Disease Control	80,000	
VIIA	Screening R&D	8,000	
Illinois Institute of Technology Research Institute (67-1141)			
IIID	Toxicology & Pharmacology		85,000
International Business Machines Corporation (64-100)			
IVA	Patient Supportive Care		320,000
International Business Machines Corporation (68-968)			
IVA	Patient Supportive Care		4,000
Jackson Laboratory (68-35)			
VA	Animal Procurement		249,000
Japanese Cancer Association (66-77)			
VIC	Literature, Publications & Related Activities		5,000
Kaman Nuclear Corporation (68-)			
IVC	Clinical Trials (Miscellaneous)		31,000

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Kansas, University of (64-64)			$ 66,000
VA	Animal Production	48,000	
VC	Trust Stocks & Genetic Control	18,000	
Kentucky, University of (67-1173)			
IVB	Clinical Experimental Therapeutics		(14,000)[1]
Laboratory Supply Company (68-1)			
VA	Animal Production		20,000
Laboratory Supply Company (68-2)			
VA	Animal Production		33,000
Laboratory Supply Company (68-19)			
VA	Animal Production		67,000
Laboratory Supply Company (68-681)			
VA	Animal Production		70,000
Laboratory Supply Company (68-956)			
VA	Animal Production		51,000
Lakeview Hamster Colony (68-26 & 68-638)			
VA	Animal Production		24,000
Leasco Systems and Research Corporation (68-30)			483,000
VIA	Data Processing	423,000	
VIC	Literature, Publications & Related Services	60,000	
Little, Arthur D., Inc. (65-61)			1,450,000
IIC	Natural Product Assay	40,000	
IIIA	Primary *In Vivo* Screening	120,000	
IIIB	*In Vitro* Screening	20,000	
IIIC	Detailed Evaluation of New & Old Drugs	173,000	
IIID	Toxicology & Pharmacology	217,000	
VD	Tumor & Cell Culture Banks	43,000	
VIA	Data Processing & Analysis	50,000	
VIIA	Screening R&D	296,000	
VIIC	Pharmacology & Physiological Disposition	491,000	
Little, Arthur D., Inc. (66-502)			
IB	New Synthesis		54,000
Litton Industries, Inc. (68-)			
IVC	Clinical Trials (Miscellaneous)		199,000

TABLE III (Continued)

CONTRACTOR		FUNCTIONS		DOLLAR LEVEL
Makerere University (67-1343)				
	IVB	Clinical Experimental Therapeutics		$ 50,000
Marshall Research Animals, Inc. (68-39)				
	VA	Animal Production		16,000
Mason Research Institute (65-51)				
	IIID	Toxicology & Pharmacology		199,000
Medical Literature, Inc. (67-1353)				
	VIC	Literature, Publications & Related Activities		4,000
Merck and Company (62-479)				426,000
	IA1	Clinical Preparative Laboratories	226,000	
	IA2	Non-Clinical Preparative Laboratories	200,000	
Miami, University of (64-890)				57,000
	IIIB	*In Vitro* Screening	52,000	
	VIIA	Screening R&D	5,000	
Miami, University of (67-1179)				
	IIB	Plant & Other Natural Prod. Coll. & Isolation		29,000
Michigan Department of Health (65-7)				
	IIC	Natural Product Assay		32,000
Michigan, University of (65-45)				
	VIB	Statistical Services (Clinical)		9,000
Michigan, University of (68-)				
	IC	Quality Control, Dosage Form., etc.		69,000
Michigan, University of (68-)				
	IB	New Synthesis		48,000
Microbiological Associates, Inc. (62-161)				
	VA	Animal Production		320,000

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Microbiological Associates, Inc. (64-911)			$ 1,180,000
IIIA	Primary Screening	55,000	
IIIC	Detailed Evaluation of New & Old Drugs	370,000	
IIID	Toxicology & Pharmacology	120,000	
VD	Tumor & Cell Culture Banks	25,000	
VIIA	Screening R&D	190,000	
VIIC	Pharmacology & Physiological Disposition	250,000	
VIID	Immunity & Immune Response	170,000	
Microbiological Associates, Inc. (65-587)			350,000
IB	New Synthesis	215,000	
IC	Quality Control, Dosage Form., etc.	135,000	
Midwest Research Institute (65-94)			
IB	New Synthesis		321,000
Millerton Research Farm, Inc. (68-691)			
VA	Animal Production		28,000
Monsanto Research Corporation (65-1044)			126,000
IA1	Clinical Preparative Laboratories	110,000	
IA2	Non-Clinical Preparative Laboratories	16,000	
Monsanto Research Corporation (67-658)			145,000
IA1	Clinical Preparative Laboratories	125,000	
IA2	Non-Clinical Preparative Laboratories	20,000	
National Academy of Sciences (64-44) (Task Order #6)			
VB	Animal Disease Control		15,000
National Academy of Sciences (64-44) (Task Order #16)			
VID	Research Services (Miscellaneous)		18,000
National Academy of Sciences (64-44) (Task Order #19)			
VID	Research Services (Miscellaneous)		11,000
National Academy of Sciences (64-44) (Task Order #41)			
VID	Research Services (Miscellaneous)		13,000
National Bureau of Standards (68-45)			
VIC	Literature, Publications & Related Activities		14,000
National Educational Television (67-1196)			
VID	Research Services (Miscellaneous)		30,000

TABLE III (Continued)

CONTRACTOR		FUNCTIONS		DOLLAR LEVEL
New Jersey College of Medicine & Dentistry (66-52)				
	VB	Animal Disease Control		$ 111,000
New York University (67-1174)				
	IVB	Clinical Experimental Therapeutics		11,000
New York University (67-1336)				
	VIA	Data Processing		(60,000)1/
Nuclear Services Corporation (68-)				
	IIB	Plant & Other Natural Prod. Coll. & Isolation		30,000
Ohio State University (67-1175)				
	IVB	Clinical Experimental Therapeutics		(13,000)1/
Parke, Davis & Company (4320)				(92,000)1/
	IA1	Clinical Preparative Laboratories	(32,000)	
	IA2	Non-Clinical Preparative Laboratories	(60,000)	
Perkin-Elmer Corporation (66-1159)				
	IVC	Clinical Trials (Miscellaneous)		12,000
Pfizer, Charles and Company, Inc. (67-687)				
	IC	Quality Control, Dosage Form., etc.		250,000
Pfizer, Charles and Company, Inc. (68-45)				1,300,000
	IB	New Synthesis	25,000	
	IIA	Antibiotic Fermentation & Isolation	180,000	
	IIB	Plant & Other Natural Prod. Coll. & Isol.	203,000	
	IIC	Natural Product Assay	225,000	
	IID	Production for Pharmacology & Clinic	125,000	
	IIIC	Detailed Evaluation of New & Old Drugs	65,000	
	IIID	Toxicology & Pharmacology	165,000	
	IVC	Clinical Trials (Miscellaneous)	51,000	
	VA	Animal Production	95,000	
	VIIA	Screening R&D	52,000	
	VIIC	Pharmacology & Physiological Disposition	114,000	
Pico Laboratories, Inc. (67-1167)				
	IIID	Toxicology & Pharmacology		35,000
Pittsburgh, University of (67-1186)				
	IC	Quality Control, Dosage Form., etc.		(22,000)1/

TABLE III (Continued)

CONTRACTOR		FUNCTIONS		DOLLAR LEVEL
Public Health Service Division of Hospitals (Transfer -64-3)				
	IVB	Clinical Experimental Therapeutics		$ 1,381,000
Rawley Farms (68-625 & 68-703)				
	VA	Animal Production		123,000
Research Triangle Institute (4322)				
	IIB	Plant & Other Natural Prod. Coll. & Isolation		123,000
Roma Kennels (68-3)				
	VA	Animal Production		9,000
Schmidt, A. R., Company (66-86)				
	VC	Trust Stocks & Genetic Control		78,000
Schmidt, A. R., Company (66-484)				95,000
	VA	Animal Production	65,000	
	VC	Trust Stocks & Genetic Control	30,000	
Schmidt, A. R., Company (66-901)				
	VC	Trust Stocks & Genetic Control		39,000
Schmidt, A. R., Company (67-110)				130,000
	VA	Animal Production	98,000	
	VC	Trust Stocks & Genetic Control	32,000	
Schmidt, A. R., Company (68-21)				
	VA	Animal Production		88,000
Schmidt, A. R., Company (68-682)				
	VA	Animal Production		66,000
Scripps Clinic and Research Foundation (65-14)				
	VIIB	Biochemical Mechanisms		54,000
Simonsen Laboratories, Inc. (65-55)				62,000
	VA	Animal Production	48,000	
	VC	Trust Stocks & Genetic Control	14,000	
Simonsen Laboratories, Inc. (68-32)				
	VA	Animal Production		20,000
Simonsen Laboratories, Inc. (68-698)				
	VA	Animal Production		87,000

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Southern Animal Farms (68-31)			
VA	Animal Production		$ 35,000
Southern Animal Farms (68-701)			
VA	Animal Production		51,000
Southern Research Institute (63-68)			
VIIA	Screening R&D		5,000
Southern Research Institute (64-51)			
IB	New Synthesis		388,000
Southern Research Institute (65-594)			1,400,000
IIC	Natural Product Assay	50,000	
IIIA	Primary In Vivo	350,000	
IIIC	Detailed Evaluation of New & Old Drugs	665,000	
VD	Tumor & Cell Culture Banks	30,000	
VIIA	Screening R&D	275,000	
VIIB	Biochemical Mechanisms	30,000	
Southern Research Institute (65-654)			
VIIC	Pharmacology & Physiological Disposition		222,000
Southern Research Institute (66-29)			224,000
VIIA	Screening R&D	50,000	
VIIB	Biochemical Mechanisms	174,000	
South Shore Analytical & Research Laboratory, Inc. (64-932)			
IIID	Toxicology & Pharmacology		165,000
Squibb, E. R. & Sons (65-1056)			403,000
IIA	Antibiotic Fermentation & Isolation	103,000	
IID	Production for Pharmacology & Clinic	300,000	
Stanford Research Institute (64-500)			558,000
IB	New Synthesis	380,000	
IC	Quality Control, Dosage Form., etc.	178,000	
Stanford Research Institute (65-575)			
VIIB	Biochemical Mechanisms		116,000
Stanford Research Institute (67-1143)			
IIID	Toxicology & Pharmacology		(80,000)[1/]

TABLE III (Continued)

CONTRACTOR		FUNCTIONS		DOLLAR LEVEL
Stanford Research Institute (68-)				
	IA2	Non-Clinical Preparative Laboratories		$ 172,000
Starks Associates, Inc. (62-484)				169,000
	IA1	Clinical Preparative Laboratories	79,000	
	IA2	Non-Clinical Preparative Laboratories	90,000	
Starks Associates, Inc. (68-)				
	IA2	Non-Clinical Preparative Laboratories		72,000
Taconic Farms, Inc. (68-695)				
	VA	Animal Production		65,000
Texas Inbred Mice Company (68-88)				220,000
	VA	Animal Production	172,000	
	VC	Trust Stocks & Genetic Control	48,000	
Texas Inbred Mice Company (68-696)				
	VA	Animal Production		39,000
Texas Inbred Mice Company (68-962)				
	VA	Animal Production		40,000
Texas, University of (66-1156)				
	VIIC	Pharmacology & Physiological Disposition		200,000
Tulane University (65-593)				
	VC	Trust Stocks & Genetic Control		(83,000)1/
Tyco Laboratories, Inc. (67-723)				
	IIB	Plant & Other Natural Prod. Coll. & Isolation		32,000
Upjohn Company (62-168)				517,000
	IIA	Antibiotic Fermentation & Isolation	158,000	
	IIC	Natural Product Assay	12,000	
	IID	Production for Pharmacology & Clinic	52,000	
	IIIB	*In Vitro* Screening	68,000	
	VIIA	Screening R&D	18,000	
	VIIB	Biochemical Mechanisms	65,000	
	VIIC	Pharmacology & Physiological Disposition	144,000	
Utah, University of (65-1041)				
	IB	New Synthesis		128,000
Veterans Administration (Transfer - 64-4)				
	IVB	Clinical Experimental Therapeutics		1,000,000

TABLE III (Continued)

CONTRACTOR		FUNCTIONS		DOLLAR LEVEL
Vinemont Animal Farms (68-655)				
	VA	Animal Production		$ 28,000
Wake Forest University (68-950)				
	IVB	Clinical Experimental Therapeutics		6,000
Walter Reed General Hospital (Transfer - 64-5)				
	IVB	Clinical Experimental Therapeutics		42,000
Wisconsin Alumni Research Foundation (66-494)				292,000
	IIC	Natural Product Assay	100,000	
	IIIA	Primary *In Vivo* Screening	140,000	
	IIIC	Detailed Evaluation of New & Old Drugs	45,000	
	VIIA	Screening R&D	7,000	
Wisconsin Alumni Research Foundation (67-747)				
	IIB	Plant & Other Natural Prod. Coll. & Isolation		50,000
Wisconsin, University of (64-551)				
	IIB	Plant & Other Natural Prod. Coll. & Isolation		90,000
Wisconsin, University of (67-1126)				
	IIC	Natural Product Assay		20,000
Yale University School of Medicine (67-1193)				
	IIIB	*In Vitro* Screening		(13,000)[1]
Zucca Hamstery (68-27)				
	VA	Animal Production		18,000
Zucca Hamstery (68-637)				
	VA	Animal Production		17,000

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[1] Not included in dollar totals since no Fiscal Year 1968 funds were obligated; included here for reference since it is an on-going contract.

TABLE IV
DESCRIPTION OF CONTRACTS
IN THE CHEMOTHERAPY PROGRAM

AGRICULTURE, U.S. DEPARTMENT OF (TRANSFER OF FUNDS)

The U.S.D.A. collects approximately 2,000 new plant materials per year for screening. Although the majority of plants are collected in this country, samples are obtained from all parts of the world.

The plants collected are shipped to the extraction contractor, Wisconsin Alumni Research Foundation. As confirmed active plants appear, the U.S.D.A. re-collects in large quantity at the rate of about 100 re-collections per year in order to provide material for the fractionating chemists.

ALDRICH CHEMICAL COMPANY (PH43-67-1471)

This contract provides for the procurement of 239 selected chemical compounds. The materials were obtained in 1-100 gram quantities primarily for drug evaluation studies. The compounds were purified, crystalline materials isolated from natural sources (plants) and related substances. This contract terminates June 30, 1968.

AMERICAN CANCER SOCIETY (PH43-68-51)

This contract provided funds for partial support for the Conference on Acute Leukemia and Burkitt's Tumor held in Boston, Massachusetts, September 20, 1967. This contract terminated December 31, 1967.

AMERICAN NATIONAL RED CROSS (PH43-64-102)

The Red Cross supplies the Medicine Branch and National Cancer Institute with 120-150 units of platelet concentrates (fresh and frozen) and approximately 100-120 units of platelet-rich plasma per week for experimental use on the clinical wards. Various techniques of platelet preservation by freezing methods have been evaluated by the use of *in vitro* tests of viability such as serial clot retraction, ADP-induced aggregation, and the release of C^{14}-serotonin. A number of plastic materials and container types have been evaluated for platelet preservation and storage. *In vivo* testing of cryo-preserved platelets is scheduled for this current year.

APPLIED RADIATION CORPORATION (PH43-67-725)

The Medical Research Linear Accelerator is under construction for the Radiation Branch, National Cancer Institute. This unit will provide a modern radiation facility required for both service work and clinical and experimental research. The Applied Radiation Corporation in Walnut Creek, California has been awarded the contract, and delivery is anticipated in August 1968.

ARIZONA, UNIVERSITY OF (PH43-67-1484)

This contract has as its major objective the fractionation of confirmed active plants in order to isolate in a pure state and identify the active components. The contractor has a number of plant materials which are or have been under fractionation and one material isolated, a glyco-protein, has been selected as a potential candidate for clinical trial. Other materials of considerable interest are nearing the final steps of purification. The majority of plant materials for use in fractionating work are obtained through another phase of this contract devoted to the re-collection of active plant materials, primarily in the Southwestern part of the country.

BATTELLE MEMORIAL INSTITUTE (PH43-62-488)

The contractor maintains a genetic-production center for inbred rodents. It provides breeding animals for large-scale laboratory rodent production colonies, and limited numbers of these animals for testing and research programs. Its profile of categories of these animals includes nine strains and three hybrids of inbred strains.

BATTELLE MEMORIAL INSTITUTE (PH43-62-495)

This contract is for the performance of *in vivo* tests of new synthetic materials and natural products, and the related secondary evaluation of active agents. At present, screening is being conducted at a level of approximately 14,000 to 17,000 tests per annum. The testing of new materials in FY 1968, performed in accordance with CCNSC screening protocols, has utilized the Lymphoid Leukemia 1210 in mice and the Walker 256 Carcinosarcoma (IM) tumor in rats. The assay systems used however, are determined by the requirements of the Chemotherapy Program, NCI, and may be changed when specifically requested by the Project Officer. Other tumor test systems are available but are reserved for special purposes and used only when requested by CCNSC. Active agents are examined for the influence of the treatment schedule and route of administration on their effectiveness and host toxicity. Methodology and/or research and development is carried out only at the request of the Project Officer and during this year has been limited to a few special studies because of the need for presumptive screening test capacity.

BATTELLE MEMORIAL INSTITUTE (PH43-63-18)

The contractor performs bacteriologic diagnostic services with emphasis upon the detection of Salmonella spp. and Pseudomonas spp. in program rodents, and other materials used for animal husbandry practices. Specimens are forwarded by contract laboratories and animal production establishments. Approximately 10,000 samples are screened per year. The research portion of this contract (a minor component) is directed towards improvement in cultural techniques, and the elucidation of salmonella inhibitory substances associated with certain microorganisms.

BELLAIRE ACRES (PH43-68-683) (formerly PH43-67-713)

This procurement contract provides for the supply of 130,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for the CCNSC compound evaluation program. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

BEN VENUE LABORATORIES, INC. (PH43-66-909)

This contract is designed to provide a pharmaceutical pilot plant for the development, formulation, and production of parenteral clinical dosage forms of antitumor agents. Specifically, the contractor performs the following duties:

1. Solubility and stability studies on chemical agents prior to development of a specific dosage form;

2. Development and production of parenteral dosage forms for clinical evaluation;

3. Stability surveillance of all dosage forms prepared.

Ben Venue Laboratories completed 31 production projects for a total of 94,578 vials during calendar year 1967.

BERKELEY-PACIFIC LABORATORIES (PH43-68-22) (formerly PH43-66-930)

This contract furnishes 52,000 random-bred rats for drug evaluation studies. Breeding animals with defined microflora are provided by the Government. The parents of the breeding rats are produced by means of the hysterotomy-isolator techniques, and originate from a prime center.

BRISTOL LABORATORIES (PH43-64-1159)

This contract covers a comprehensive effort to obtain new antitumor agents from fermentation broths or other natural products. It includes (1) the preparation of broths, or other natural products, for screening against *in vivo* systems specified by NCI, (2) a rodent tumor laboratory for *in vivo* screening and also testing of fractionation and other follow-up studies, (3) the isolation work required to obtain purified agents, (4) development and use of *in vitro* correlative assays for more rapid follow-up of such samples, (5) production of large quantities of agents approved for clinical trials, and (6) the preparation of appropriate dosage forms of such agents.

Two of Bristol's antibiotics, A-649 and Actinogan, have been in clinical study. Actinogan was dropped because of toxicity and A-649 was dropped because of inactivity in the tumors studied. The latter is being reconsidered because of its relationship to Mithramycin and its activity against the L1210 and P388 tumor systems.

A new antibiotic, Neocarzinostatin, has been formulated for clinical study and toxicology studies are nearing completion.

BURROUGH BROTHERS PHARMACEUTICALS, INC. (PH43-65-2)

This contract is concerned with the preparation of oral dosage forms of antineoplastic drugs. The contract scope includes: development and production of tablets and capsules; special labeling and coding of drugs for double-blind studies; monitoring two-year shelf-life stability studies on some formulations; and storage and distribution of "slow-usage" clinical drugs. The laboratory facilities are adequate to assure that all drugs prepared meet the established standards of safety, identity, quality and purity set forth by the Food and Drug Administration and other governing bodies.

Burrough Brothers completed 64 projects during calendar year 1967, involving the manufacture and packaging of 1,837,380 compressed tablets and 140,360 capsules.

BUTTERFLY ART JEWELRY, INC. (PH43-68-983)

This contract provides for the collection of up to 450 species of insects in broad categories, from Taiwan, as raw source materials for the preparation of extracts for screening. Extracts will be prepared by Prof. George R. Pettit of Arizona State University. Those that show reproducible antitumor activity will be re-collected and fractionated for isolation of the active agent(s).

This contract will provide entry into a large and unexplored field of natural products known to contain physiologically active substances.

CANCER RESEARCH INSTITUTE (PL-480 - Agreement No. 434310)

This contract calls for the establishment and operations of an *in vivo* screening laboratory for testing materials, both synthetics and natural products, available in India.

CENTRAL DRUG RESEARCH INSTITUTE (PL-480 - Agreement No. 134304)

Central Drug Research Institute, Lucknow, India, collects native Indian medicinal plants, supplies extracts for screening by CCNSC, and fractionates the active extracts under a PL 480 agreement. At present, 16 plants are under fractionation.

CHARLES RIVER BREEDING LABORATORIES, INC. (PH43-66-87)

This contract has as its objectives the development of germ-free foundation colonies of inbred rodents required for program studies. Pedigreed animals are derived via hysterotomy and foster-suckled in germ-free isolators. Selected pedigreed offspring are artifically contaminated with pure cultures of organisms and are developed as pedigreed expansion colonies in jacket isolators. Offspring from this second stage are issued to secondary genetic centers, who in turn produce breeding animals for large-scale production colonies. Classic methods for the maintenance of the animals are followed with respect to environmental controls and microbiological monitoring. A small-scale production colony is maintained in order to provide limited numbers of rodents for evaluation studies.

CHARLES RIVER BREEDING LABORATORIES, INC. (PH43-68-20) (formerly PH43-66-925)

This contract furnishes 93,000 random-bred rats for compound evaluation studies. Breeding animals with defined microflora are provided by the Government. The parents of the breeding rats are produced by means of the hysterotomy-isolator techniques.

CHARLES RIVER BREEDING LABORATORIES, INC. (PH43-68-87)

This procurement contract supplies 41,000 Fischer 344 rats yearly for rodent tumor tests. Original breeding animals were derived by hysterotomy and isolator techniques in order to reduce disease levels by eliminating those of major importance.

CHEMICAL ABSTRACTS SERVICE (PH43-65-1021)

The Chemical Abstracts Service is developing an automated NCI chemical registry system. The data banks in this system contain information on compounds submitted to CCNSC for testing and on other selected compounds of interest; the information included consists of chemical structure representation, nomenclature, and bibliographic references. Much of the effort of preparing the machine-processable files has been completed. The magnetic tape files will be used to determine duplicate compounds, to carry out substructure searches, and to prepare various cross-reference listings such as name to NSC number and molecular formula to NSC number.

CHEMORE CORPORATION (PH43-67-1358)

Daunomycin Hydrochloride is an antibiotic of clinical interest. The source of this material is Farmitalia, whose U. S. representative is the Chemore Corporation. Because of NCI's interest in this drug, a purchase contract was set up for the procurement of one kilogram (800 grams of the formulated material and 200 grams of bulk material) of Daunomycin Hydrochloride. This contract will terminate June 1, 1968.

CHICAGO, UNIVERSITY OF (PH43-67-1323)

This contract supports the establishment and operation of a Pathology Reference Center and Repository and six subcenters. The purpose of the Center is to maintain a permanent file of histopathologic sections on patients treated by members of the Lymphoma Task Force. Diagnostic criteria in diseases of a controversial nature are to be established and classification of the malignant lymphomas is to be clarified. Cases that present diagnostic difficulties or are controversial with respect to classification are reviewed.

CHILDREN'S CANCER RESEARCH FOUNDATION (PH43-66-541)

The activities on this research contract are aimed at establishing, as far as possible, a rational approach to clinical chemotherapy. This is accomplished by using and applying the latest concepts in Pharmacology as related to the biochemical and physiological properties of malignant tumors. When feasible the findings made in experimental models are applied to clinical situations. A good example of this aspect of the work will be the clinical trial of fluorouridine in leukemic patients after it was found to be readily phosphorylated by leukemic cells whereas fluorouracil and fluorodeoxyuridine are not phosphorylated to a significant extent. The collection program, which constitutes about one-third of the effort on this contract, collects, classifies and distributes normal and/or malignant human tissues needed for the contractor's own programs and also provides a similar service for other appropriate projects throughout the country.

CHILDREN'S CANCER RESEARCH FOUNDATION (PH43-66-1147)

This contract provides 6000 - 8000 units of platelet concentrates for clinical use at the Children's Cancer Research Foundation and elsewhere in the Boston area. In the coming year, major emphasis will be placed on platelet preservation (with DMSO) and in the provision of approximately 4000 units of preserved platelets for evaluation by members of the Acute Leukemia Task Force.

CHILDREN'S HOSPITAL OF PHILADELPHIA (PH43-66-1152)

This contractor is a major supplier of platelet concentrates for patients at the Children's Hospital of Philadelphia and other institutions in the Philadelphia area. Concentrates are occasionally supplied to other hospitals on request. Approximately 8000 - 9000 units are prepared annually. Major emphasis is now being placed on the provision of approximately 4000 units of cryopreserved platelets (5% DMSO and 5% dextrose) for evaluation by the Acute Leukemia Task Force.

COLLABORATIVE RESEARCH, INC. (PH43-66-506)

The major objective of this contract is the production of homofolic acid (NSC-79249) and/or tetrahydrohomofolic acid (NSC-89473) in kilogram quantities. Tetrahydrohomofolic acid is needed in significant quantities for preclinical pharmacological evaluation. Development work on the large-scale preparation of this substance has also been done under this contract.

In addition, a portion of this contract has been devoted to the identification of the therapeutically effective metabolite of Cytoxan and the preparation of hydrolytic products of cyclophosphamide. Since Cytoxan is not an active compound itself, its biological activation and the products therefrom are a question of practical as well as theoretical importance.

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION (PH43-64-522)

This contract provides for (1) the fractionation, and isolation of active agents contained in native plants from Australia and New Guinea submitted originally by CSIRO and found by CCNSC to be active; (2) the synthesis of such active agents, and their derivatives and analogs; and (3) the development of production methods for such active agents.

Many active plants have been found to date. A large proportion of these are presently under fractionation and several have yielded active products which are under study. One of these, ellipticine, is being synthesized in the U. S. in quantity for clinical studies under another (bulk chemical) contract, while another, acronycine, is being prepared on a large scale by extraction for similar purposes.

CONNECTICUT, UNIVERSITY OF (PH43-65-1035)

The objectives of this contract is to synthesize some pyrimidine and purine derivatives which may have selective cytotoxic effects. The principal series of agents are to be trinucleoside phosphates in which three natural or analog bases are combined with ribose or other sugars which are attached to a single phosphate. This will yield an electrophoretically neutral molecule which should be taken into cells. By this means it may be possible to introduce bases which are not normally transported into cells, as well as to by-pass some types of drug resistance.

Once prepared, the agents are to be evaluated biochemically to learn whether they are substrates for or inhibitory to important nucleic acid anabolic enzymes, and their effects in sensitive and resistant cell cultures. On the basis of this information, promising agents will be prepared in sufficient quantity for *in vivo* evaluation.

CONTROL DATA CORPORATION (PH43-67-1356)

Clinical patient research data files are being maintained and queried by use of INFOL (Information Oriented Language). TWX Communication is also being used for transmitting data for the files above and for producing card input to other systems.

CORNELL UNIVERSITY MEDICAL COLLEGE (PH43-67-1337)

The primary objectives are to develop, refine and evaluate mathematical decision-making models useful in cancer patient diagnosis, treatment planning, and subsequent clinical management. Promising techniques and models are being tested and compared under hospital conditions with conventional modes of decision-making at the Cornell University Medical Center. Considerable attention is also being directed toward data capture.

COSTA RICA, UNIVERSITY OF (PH43-64-31)

The contract is concerned with the collection of native Costa Rican plants and preparation of extracts for testing by CCNSC.

Aqueous-alcoholic extracts of native plants with reputed medicinal properties are prepared and sent for testing by CCNSC. Plants whose extracts possess reproducible activity are re-collected in large quantity and fractionation is being undertaken in one of our cooperating laboratories.

DUBLIN LABORATORY ANIMALS (PH43-68-23) (formerly PH43-66-922)

This contract furnishes 66,000 random-bred rats yearly for drug evaluation studies. Breeding animals with defined microflora are provided by the Government. The parents of the breeding rats are produced by means of the hysterotomy-isolator techniques.

DUBLIN LABORATORY ANIMALS (PH43-68-680) (formerly PH43-67-714)

This procurement contract provides for the supply of 130,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for CCNSC compound evaluation studies. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

ENGLE'S HAMSTERY (PH43-68-28)

This contract provides 26,000 hamsters produced in a controlled environment. Breeding animals raised in isolators are furnished by the Government. The parents of the breeding animals are produced under environmental controls that serve to restrict the profile of intestinal microflora and viral agents. The hamsters produced under the terms of this contract are furnished for compound evaluation studies.

GEORGE WASHINGTON UNIVERSITY (PH43-67-1338)

The purpose of this project is to facilitate scanning and hard copy retrieval of cancer chemotherapy literature by incorporating into an automatic microfilm reader-printer system the documents and/or abstracts comprising essentially those cited in Cancer Chemotherapy Abstracts, (CCA), Volume 1, 1960 through Volume 7, 1966, and further, to provide both cumulative agent and author indexes.

HARLAN INDUSTRIES, INC. (PH43-68-957)

This procurement contract is designed to furnish yearly 78,000 six-week old CDF_1 (BALB/c♀ x DBA/2♂) hybrid mice free of pseudomonas for CCNSC contract studies. Breeding animals originate from genetic centers. The mice are offered chlorinated water in order to eliminate or control the incidence of pseudomonas.

HARVARD COLLEGE (PH43-65-589)

This contract provided for the study of substances in plasma, urine, and spinal fluid which might be used to detect residual leukemic and malignant cells in patients after therapy. Studies of trace metals and enzymes were of major interest. Urinary zinc excretion and serum zinc levels appeared to be of limited significance in the evaluation of patients with acute leukemia and Hodgkin's disease. An inhibitor of the enzyme lactic dehydrogenase found in the urine did not prove useful as an indicator of the presence of a relatively small number of leukemic cells. Serum copper determination was of limited value in detecting residual disease in patients with acute leukemia in remission. This contract will terminate at the end of the current period.

HAWAII, UNIVERSITY OF (PH43-67-748)

This contract provides for the collection of a minimum of 500 plant samples from among the indigenous and exotic higher plants of the Hawaiian Islands and the re-collection of larger samples of those that are reproducibly active in the CCNSC tests.

Plants so obtained are shipped to the extraction contractor, Wisconsin Alumni Research Foundation, and the extracts tested at one of our contract screeners.

HAZLETON LABORATORIES, INC. (PH43-63-622)

This contract is for the performance of *in vivo* screening of new materials and limited secondary evaluation of active materials. Testing is currently being carried out at a level of approximately 30,000 tests per contract year. During FY 1968, routine primary screening has been conducted against two test systems: Lymphoid Leukemia L1210 (3LE) in mice, and intramuscularaly-implanted Walker carcinosarcoma W-256 (5WM) in rats. Testing is performed according to CCNSC screening protocols.

Secondary evaluation consists primarily of limited investigations, requested by CCNSC staff, of the influence of treatment schedules and treatment routes on drug activity.

Methodology and/or research and development is devoted to specific problems that arise in the routine screening.

HAZLETON LABORATORIES, INC. (PH43-63-1168)

The purpose of this contract is the evaluation of animal toxicity of potential cancer chemotherapeutic agents using most frequently mice, rats, dogs, and monkeys. Evaluation is based on lethality, hematology, clinical and analytical chemistry, gross pathology, light and electron microscopic examination and other parameters as dictated by the nature of and past experience with a given compound or its analogs. Results of observations are subjected to statistical analysis where appropriate and reported to the Laboratory of Chemical Pharmacology, NCI.

Increasing emphasis is being given to the effects of dosage spacing and of drug combinations on animal toxicity.

HAZLETON LABORATORIES, INC. (PH43-66-84)

The purpose of this contract is to explore the feasibility of developing model tumor systems in primates. Such a system can then be used for the study of the pharmacology and experimental therapeutics of drugs of clinical interest in tumor-bearing primates. Currently, monkeys with primary liver tumor induced by *N*-nitrosodiethylamine are being used in chemotherapy experiments and in liver enzyme studies. A tissue culture line NCLP-6, derived from a primary induced hepatocarcinoma, will grown on intracerebral homologous transplantation and provides a unique test system to study drug distribution and the therapeutic effect of combined surgical and chemotherapeutic procedures on tumors growing in the brain. It should be noted that information is being obtained on the carcinogenicity in non-human primates for a variety of materials carcinogenic in rodents and suspect of carcinogenicity in man. Although such information is not immediately pertinent to the chemotherapy area, it is of considerable importance to cancer research in general.

HEALTH RESEARCH, INC. (PH43-64-549)

Acute Leukemia Group B received statistical consultation and services from this contract. Additional work carried out comprised processing of clinical records; reduction of data to machine-processable form and analysis of results which are presented at group meetings; submission of punched cards for the Uniform Reporting System; and assistance to the group chairmen and investigators in the design of studies and formulation of protocols. This contract terminated October 31, 1967.

HEALTH RESEARCH, INC. (PH43-65-44)

This contract is designed to monitor and maintain genetic control of tumor strains and inbred mouse stocks. This service was established to assure continuous control of the biological materials used in program studies. The contractor carries out the service by: (1) performing iso-antigenic typing tests of tumors used in the various screening tests, enabling CCNSC to maintain and distribute tumors identical to the prototype; (2) developing techniques and material for antigenic typing of tumors new to the screening program; (3) determining strain specificity of tumors originating in the inbred mouse; and (4) performing skin grafts and antigenic studies of mouse strains to assure their continuous genetic integrity and histocompatibility with other sublines maintained in counterpart genetic-production centers.

ILLINOIS INSTITUTE OF TECHNOLOGY RESEARCH INSTITUTE (SA-43-ph-4349)

This contract is for the performance of *in vivo* testing of new synthetic materials and natural products, and the related secondary evaluation of active agents. Screening is being conducted at a level of approximately 16,000 tests per annum. This testing of new materials, performed in accordance with CCNSC screening protocols, utilized during FY 1968 the Lymphoid Leukemia 1210 in mice and the Walker 256 Carcinosarcoma (IM) tumor in rats. The assay systems used however, are determined by the requirements of the Chemotherapy Program, NCI, and may be changed when specifically requested by the Project Officer. Other tumor test systems are available but are reserved for special purposes and used only when requested by CCNSC. Active agents are examined for the influence of the treatment schedule and route of administration on their effectiveness and host toxicity.

This laboratory also maintains a routine Salmonella spp. and Pseudomonas spp. diagnostic laboratory and provides general animal disease diagnostic services as requested. These studies employ virology, bacteriology, pathology, and histopathology. In addition, it explores routine tumor growth characteristics in germfree, gnotobiotic and conventional animals.

Methodology or research and development is carried out only at the request of the Project Officer and during this year has been devoted to specific problems that arise in the *in vivo* screening program and the development of their viral disease diagnosis capability and germfree animal studies.

ILLINOIS INSTITUTE OF TECHNOLOGY RESEARCH INSTITUTE (PH43-67-724)

This contract, which started the latter part of FY 1967, provided for the preparation of a number of pure cultures of algae in sufficient quantities to provide two specified samples from each culture. These samples (ethanol:water extracts) are screened for *in vivo* activity in animal tumor systems. This contract terminates late in FY 1968.

ILLINOIS INSTITUTE OF TECHNOLOGY RESEARCH INSTITUTE (PH43-67-1141)

The primary objective of this contract is to investigate the influence of dosage schedule on the toxicity of established and investigational cancer chemotherapeutic agents. A cytokinetic profile of organs known to be damaged by the drugs under investigation is being compiled or measured; dosage regimens derived from the cytokinetic data will be tested in an animal species in an attempt to minimize the toxicity.

Three dosage regimens are being initially employed: (1) single injection, (2) spaced pulse and (3) spaced infusions.

The second objective of this contract is to develop serum chemical and biopsy procedures that will serve as more sensitive indicators of organ-specific toxicity.

INTERNATIONAL BUSINESS MACHINES CORPORATION (PH43-64-100)

A continuous flow blood cell separator has been developed with the capability of separating on a continuous in vivo basis the various cellular components of the blood. The principal efforts in the immediate future will be concerned with improved efficiency of granulocyte separation and preservation and the preparation of platelet concentrates.

JACKSON LABORATORY (PH43-68-35)

This service contract provides for the annual procurement of 92,000 C57BL/6 and 62,000 DBA/2 inbred mice to screening contractors for tumor transplantation and as breeders for large-scale hybrid production colonies. It also supplies 62,000 AKR inbred mice and a small number of BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are supplied by the contractor.

JAPANESE CANCER INSTITUTE (PH43-66-77)

The purpose of this contract is to scan, translate, and abstract Japanese language cancer literature pertinent to the interest of the Chemotherapy program. An ancillary purpose is to determine the amount of redundancy in the Japanese literature. Work on this contract will be coordinated with a similar project sponsored by the National Library of Medicine in order to prevent duplication of effort. This contract will terminate June 30, 1968.

KANSAS, UNIVERSITY OF (PH43-64-64)

This contractor maintains a genetic-production center. Its most important function is to secure the maintenance of the strains of mice that are of interest in CCNSC program investigations. Its spectrum includes six strains. Small groups of animals in excess of pedigree requirements are shipped to contractor laboratories for developmental studies. These strains include C57BL/6, DBA/2, AKR, BALB/c, C3H/He and A/He. Animals not required for perpetuation of pedigree are supplied to screening contractors and as breeders for production colonies.

KENTUCKY, UNIVERSITY OF (PH43-67-1173)

The purpose of this contract is to provide data retrieval support for experimental chemotherapy of selected patients with brain tumors in accordance with Brain Tumor Chemotherapy Study Protocol No. 6601, so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups, randomly selected.

LABORATORY SUPPLY COMPANY (PH43-68-1, PH43-68-2 and PH43-68-956)

These procurement contracts are designed to furnish yearly 130,000 six week old and 26,000 ten week old CDF_1 (BALB/c♀ x DBA/2♂) mice free of pseudomonas for CCNSC contract studies. Breeding animals are furnished by the Government. The mature mice are used mainly for spleen colony studies. Mature mice and weanlings not required for this purpose are used for the evaluation of compounds in

LABORATORY SUPPLY COMPANY (PH43-68-1, PH43-68-2 & PH43-68-956) (Cont'd.)

leukemia-related tumor systems. The mature mice are held on chlorine-water for about four weeks beyond the normal shipping time. The balance of the colony also receives the chlorine solution.

LABORATORY SUPPLY COMPANY (PH43-68-19)

This contract furnishes 74,000 random-bred rats for compound evaluation studies. Breeding animals with defined microflora are provided by the Government. The parents of the breeding rats are produced by means of the hysterotomy-isolator techniques.

LABORATORY SUPPLY COMPANY (PH43-68-681)

This procurement contract provides for the supply of 130,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for the CCNSC screening program. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

LAKEVIEW HAMSTER COLONY (PH43-68-26 and PH43-68-638)

These procurement contracts provide for the supply of 32,500 random-bred Syrian hamsters which are used for drug evaluation studies involving hamster and human tumors. Breeding animals are supplied by the contractor.

LEASCO SYSTEMS AND RESEARCH CORPORATION (formerly Documentation Inc.) (PH43-68-30)

Data processing services for the Chemotherapy program are provided by this contract with main emphasis on the reduction of data and the dissemination of information developed in the screening programs of the Drug Evaluation Branch and Endocrine Evaluation Branch, General Laboratories and Clinics, NCI. Camera-ready copy for publication is also prepared both by computer and by conventional means.

LITTLE, ARTHUR D., INC. (PH43-65-61)

This is a broad research and development program designed to examine a multitude of preclinical problems in cancer chemotherapy. During FY 1968 the contract scope has been divided into eight general areas as follows:

1. Screening and Experimental Therapeutics

2. Kinetic Studies

3. Toxicology and Pharmacology

4. Physiological Disposition and Biochemistry

5. Neuropharmacology and Viral Oncology

6. Cell Culture and Methodology

7. Cryobiology

8. Data Analysis and Operations Research

Activities in the Screening and Experimental Therapeutics area include studies to determine the antitumor activity of new and selected agents in the primary screening tumor systems which in FY 1968 were Leukemia L1210 and Walker 256 (IM). In addition the Contractor carries out special experimental studies with other selected tumor systems as may be needed by the NCI program.

Studies on the kinetics of neoplastic cell growth comprise an entire division of the contract. Past efforts have resulted in the application of the spleen colony assay procedure to L1210 and the development of background data on a number of other tumor systems. Such techniques are employed to measure cell behavior in response to drug therapy.

Preclinical toxicological studies are conducted according to protocols established by the Laboratory of Chemical Pharmacology Chemotherapy Program Staff. These are supplemented by special pharmacological studies at the request of the Experimental Therapeutics Area of the Chemotherapy Program. Biochemical studies emphasized the development and use of analytical methods for determining the physiological disposition and biochemical sites of drug action. Structure-activity studies are directed toward the association of specific toxicities to biochemical effects. Electron microscopy of pertinent lesions is also undertaken at the discretion of the appropriate LCP Project Officer in conjunction with the Head of the Laboratory of Toxicology at Arthur D. Little.

The increasing use of antineoplastic agents via the intrathecal route of administration suggested the need for more toxicological information on the effect of these agents on the central nervous system. Methotrexate, azaguanine, cytosine arabinoside and other agents, in use or under consideration for intrathecal use in man, are tested for their toxicity by this route in monkeys.

Studies are also under way to characterize and develop the brain tumors resulting from intracranial administration of Rous sarcoma virus in newborn puppies, in the hope that a model system for brain tumors in a large laboratory animal can be developed.

The Cell Culture Screening and Methodology program consists of routine cell culture testing according to standard protocols and cell kinetics studies utilizing L1210 in continuous culture. The majority of the routine *in vitro* testing is devoted to fractions of active plant materials.

The cryobiology activity provides for the maintenance of a tumor and cell bank, distribution service and appropriate methodology studies for improving the techniques used for the preservation of tumor lines.

During FY 1968 efforts were devoted to the analysis of experimental tumor data to determine if better methods of presentation and decision making could be devised.

LITTLE, ARTHUR D., INC. (PH43-66-502)

This contract is concerned with the chemical synthesis of macromolecular antimetabolites. Histones are basic proteins (or polypeptides) found in the nuclei of the cells. Recent experiments indicate that histones regulate the synthetic functions of the cell. Various histones have different inhibitory activities in regulating RNA synthesis. The contractor is preparing a number of macromolecular biodegradable polycationic compounds which may alter the synthesis in the cell nucleus by combining with DNA and/or acidic proteins and disrupt the metabolic process in the cell.

In addition, effort is being devoted to the preparation of synthetic modivications and/or derivatives of naturally occurring oxygen heterocyclic compounds; such as peucedanin, griseofulvin, flavones, and aurones.

MAKERERE UNIVERSITY (PH43-67-1343)

This contract supports the Lymphoma Treatment Centre at Makerere University, Kampala, Uganda under the immediate supervision of Dr. John Ziegler. A large group of patients with Burkitt's tumor are available in the area and studies are progressing in therapy, immune host factors, and metabolism of drugs in African patients.

MARSHALL RESEARCH ANIMALS, INC. (PH43-68-39)

This procurement contract provides 200 beagles for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the contractor.

MASON RESEARCH INSTITUTE (PH43-65-51)

This service-type contract is concerned with the toxicologic evaluation of eleven compounds in accordance with procedures delineated in the Laboratory of Chemical Pharmacology abbreviated toxicology protocols. Studies are carried out at graded dose levels in several animal species to establish the qualitative and quantitative toxicity and the maximum tolerated doses of the agents under test. Approximately 10% of the total effort is devoted to the study of metabolism and fate of selected agents as requested by the Project Officer.

MEDICAL LITERATURE, INC. (PH43-67-1353)

This contract provides for the preparation of Cancer Chemotherapy Abstracts (Volume 8, 1967), a current awareness service, which supplies abstracts and annotations of the world's literature pertinent to cancer chemotherapy. The volume is published in bimonthly issues followed by an annual cumulated index.

MERCK AND COMPANY (PH43-62-479)

This contract provides for the resynthesis of compounds, including endocrines, in amounts needed for clinical trial or for necessary preclinical evaluations. About 50% of the budget is devoted to clinical preparations.

This is the largest and oldest of the CCNSC preparative laboratories. Because of their extensive and diverse experience, the contractor is able to prepare compounds of many different and difficult types. These are materials for which no commercial source is available or which can not be prepared in the required quantities by the original supplier.

MIAMI, UNIVERSITY OF (PH43-64-890)

This contract is primarily for the *in vitro* presumptive screening of plant materials, and the follow-up testing of natural products having demonstrated activity in the CCNSC *in vivo* screening program as a tool for fractionation. Presumptive screening of synthetic materials is also carried out when the quantity of these materials is not sufficient to do primary *in vivo* screening. The procedures are those prescribed by CCNSC protocols which are based on the methods established by Eagle and Foley. Approximately five thousand tests at three to five dose levels per test are performed yearly.

Research and development studies are currently limited to the rate of drug action and the comparative cytotoxicity of drugs on cell lines derived from normal and malignant tissues.

MIAMI, UNIVERSITY OF (PH43-67-1179)

This contract covers the collection, identification and extraction of marine invertebrates for preliminary screening as potential anticancer agents. Materials displaying confirmed activity in the screening program will be recollected in large quantity and supplied to a fractionating chemist. Approximately 100 new materials will be supplied per year.

MICHIGAN DEPARTMENT OF HEALTH (PH43-65-7)

This contract provides assay services, utilizing a variety of cell culture methods, as aids in the purification of potential antitumor agents from fermentation broths. The methods employed include paper chromatography and paper electrophoresis in conjunction with cell culture agar diffusion bioautography, as well as growth inhibition assays, both in stationary and suspension cultures.

Using these methods, a number of purified materials have been submitted for consideration as potential clinical agents. Some of these are active in L1210 and some in the W256 tumor system.

The related fermentation and isolation program, serviced by this assay contract, is financed by the Michigan Department of Health. This program has provided the CCNSC with two materials (Alpha sarcin and Restrictocin) for clinical study.

MICHIGAN, UNIVERSITY OF (PH43-65-45)

This contract provides statistical services for the VA Chemotherapy Group, the VA Lung Group, and the VA Pacific Group. Work is also directed toward: processing of clinical records; reduction of data to a machine-processable form and analysis of results which are presented at group meetings; submission of punched cards to the Uniform Reporting System; and assistance to the group chairmen and investigators in the experimental design of studies and development of protocols. This contract will terminate June 30, 1968.

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-62-161)

This contract provides for the maintenance of nuclei of selected inbred strains of mice and rats; maintains a stabilization and holding area for animals received from various genetic centers and production colonies; conducts selective studies in the field of Laboratory Animal Husbandry; performs pilot production studies with unusual strains and hybrids of strains not commonly used in this manner; provides animal caretaken services for all research areas identified with this contract.

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-64-911)

Contract activities are conducted under the day-to-day guidance of the NCI Chemotherapy staff for the purpose of examining, in detail, the biological, biochemical, and pharmacological properties of materials which are uncovered in primary screens and which are of clinical interest. The program is designed to investigate, at the laboratory level, specific problems that arise in the clinic and to develop new methods for the quantitative evaluation of potential antitumor agents. These investigations are carried out in five major Divisions within the contract scope, namely, Chemotherapy Research, Drug Evaluation, Immunology, Biochemical Pharmacology and Preclinical Pharmacology.

Activities of the Chemotherapy Research Division include investigations of primary and secondary screening procedures and methodology, and detailed studies on the mode of action of drugs on tumor cells and of the influence of drugs on tumor-host interactions. Additional efforts are aimed at exploring new and potentially useful biological and biochemical methodology for prediction, evaluation and improvement of drug therapy. Special studies are conducted on the chemotherapy of selected tumor systems including virus-induced, carcinogen-induced, spontaneous and functional tumors.

The Drug Evaluation Division is concerned with studies on the quantitative evaluation of agents which emerge as active from the primary screen, in an effort to provide information applicable to the clinical use of these agents. Employing the L1210 and other in vivo systems, experiments are conducted to evaluate such factors as (a) optimal conditions for drug usage, (b) the influence of drugs on normal and neoplastic cell kinetics and (c) the possible therapeutic advantages of combination chemotherapy versus treatment with a single agent. Drug-resistant tumor variants are also utilized to evaluate the efficacy of drugs administered alone or in combination for their ability to overcome or delay resistance.

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-64-911) con't.

Efforts in the Immunology Division are directed to the search for fundamental knowledge concerning the immunology of viral oncogenesis which may lead to a better understanding of the possible control or suppression of the disease by the use of immunotherapy employed alone or in combination with anti-neoplastic drugs. Transplantation resistance procedures and immunofluorescence techniques are employed to study immunological properties of virus-induced leukemias in animals. Additional studies include the development of model systems to investigate adoptive transfer and its influence on tumor growth, and the development of systems for assessing the immuno-suppressive qualities of potential anti-neoplastic agents. This area also maintains a frozen cell and tumor repository.

The Biochemical Pharmacology and Preclinical Pharmacology Divisions are engaged in biochemical and pharmacological studies aimed at uncovering detailed knowledge of the mechanism of action of drugs which may serve to provide information of primary importance in clinical studies with such agents. Biochemical investigations include the use of enzymatic blockade and related biochemical events as indices of response to therapy, mechanism of action studies, and mechanism of resistance studies. The Preclinical Pharmacology effort is chiefly directed to the examination of the physiological distribution, toxicological and pharmacological aspects of compounds being considered for clinical trial. Attention is also devoted to more detailed examination, in laboratory animals, of specific problems which arise in the use of antitumor agents in the clinic.

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-65-587)

This contract provides a service for handling the receiving, storage, distribution and inventory records of non-clinical and bulk drugs for CCNSC. The Contractor (1) receives and maintains the chemicals and drugs in stock; (2) keeps a running inventory of all stock; (3) packs and ships the chemicals and drugs to screeners and other laboratories, at the request of CCNSC. The contract also contains a limited, but expanding effort in organic chemistry.

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-67-718)

This procurement contract provided for the supply of 104,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for the CCNSC screening program. Breeding animals originated in genetic centers and/or C57BL/6 and DBA/2 production colonies.

This contract terminated March 19, 1968.

MIDWEST RESEARCH INSTITUTE (PH43-65-94)

Midwest Research Institute is one of three basic synthetic contractors for the CCNSC. Its major objective is the rapid exploitation of leads generated by the Drug Evaluation Branch and Clinical groups. The broad experience and mature judgement in synthetic chemistry of this group to carry out such functions have been of great value to the Chemotherapy Program.

MIDWEST RESEARCH INSTITUTE (PH43-65-94) con't.

This contractor has provided a large number of compounds to CCNSC for screening. These compounds represent the exploration of a variety of leads. Current investigation centers around three areas:

1. Chemical extension of clinically significant materials: Structural modification of folic acid and related compounds; Analogs of indole alkaloids and related compounds; Structural variations of natural quinones; Synthesis of camptothecin and other natural products; and studies on tylocrebrine derivatives.
2. Analogs of biologically active agents: Thiosemicarbazone derivatives; α,β-unsaturated ketones and lactones; and 1,2,4-triazoles.
3. Extracts from Chinese medicinal plants are being prepared for antitumor evaluation.

The materials prepared have been of high purity, thoroughly characterized and in sufficient quantity for adequate evaluation.

MILLERTON RESEARCH FARM, INC. (PH43-68-691)

This procurement contract provides 52,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for screening tests. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

MONSANTO RESEARCH CORPORATION (PH43-65-1044)

This service preparative contract provides for the synthesis of radioactive labelled chemicals and drugs for use in preclinical pharmacological and clinical studies. Many of the materials prepared are not available from commercial sources. All materials, whether prepared at Monsanto or acquired from other sources, are analyzed as to radioautograph, assay, identity, etc. This contract also provides storage facilities for labelled materials and distributes such as directed by the NCI staff.

MONSANTO RESEARCH CORPORATION (PH43-67-658)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

This contract also provides for the large-scale extraction and isolation of natural products needed for preclinical and clinical studies.

NATIONAL ACADEMY OF SCIENCES (PH43-64-44 - TASK ORDER #6)

This contract Task Order serves to develop standards for animal care and maintenance; shipping standards for the various species of laboratory animals; standards for nomenclature used to identify stocks and strains of laboratory animals; standards for methodology in maintaining axenic and gnotobiotic animals; standards for animal maintenance in the research laboratory; and laboratory animal procurement standards. These standards are formulated by *ad hoc* committees whose memberships represent commercial animal production colonies, governmental and academic institutions, commercial laboratories, and non-profit research institutions.

NATIONAL ACADEMY OF SCIENCES (PH43-64-44 - TASK ORDER #16)

This contract Task Order provides for conferences on diverse subjects that are intimately associated with the field of Bio-medical Research. The agenda for a specific meeting may be concerned with a common problem, or to develop the basis for a research program that is being considered by a segment of the scientific community. Conferences in the past have been held on research facilities; the control of *P. aeruginosa*; the provision of animals for geriatrics investigations; gnotobiotic technology; and requirements for standards in specific areas. Speakers are invited to present their thoughts, and time is provided for open forum discussions. The proceedings of these conferences are published and made available to the scientific community.

NATIONAL ACADEMY OF SCIENCES (PH43-64-44 - TASK ORDER #19)

The purpose of the Task Order is to support the activity of the United States National Committee on the International Union against Cancer.

Since its inception in December, 1964, this supporting effort has included travel and subsistance and support of conferences and symposia held in various parts of the world. Planning is commencing for a conference to be held in Houston, Texas, in 1968.

NATIONAL ACADEMY OF SCIENCES (PH43-64-44 - TASK ORDER #41)

The National Cancer Institute is furnishing partial support in the amount of $12,500 for the Fifth International Congress on Photobiology, which is being organized by the Committee on Photobiology, Division of Biology and Agriculture, National Research Council.

The encouragement of basic research in radiation biology is considered to be of great programmatic interest to the Extramural Radiation Program of the National Cancer Institute. The interchange of scientific information which will take place at the Congress will stimulate future research progress in this area of direct relevance to both the etiology and treatment of cancer in man.

The proposed Congress is the fifth in a series established by the Comite International de Photobiologie, which has as its stated purpose the stimulation of scientific research in: (a) physics, chemistry, and climatology of non-ionizing radiations in relation to their biological effects, and (b) the effects of these radiations when applied in biology and medicine.

The topics for discussion at several of the lectures and symposia are directly related to cancer research. These include lectures on Basic Photochemistry, Radiation Damage and Repair *in vivo*, and Effects of UV on Skin, and symposia on Basic Photochemistry, Instrumentation and Action Spectroscopy, and Photochemistry of Macromolecules.

NATIONAL BUREAU OF STANDARDS (PH43-68-45)

Modifications are being made to the Microcite, a device built by NBS for use in the Scientific Records Section's chemical information storage and retrieval system. The engineering changes will effect direct readout of an NSC number on an IBM card suitable for computer entry. Also, chemistry cards for 36,000 compounds were microfilmed with NBS technical assistance, and additional microfilm matrices are in process.

NATIONAL EDUCATIONAL TELEVISION (PH43-67-1196)

This contract financed the production of two documentary films on the Chemotherapy Program, "The Search" and "The Battle in the Cell". Both films are being shown throughout the National Educational Television Network.

NEW JERSEY COLLEGE OF MEDICINE AND DENTISTRY (PH43-66-52)

This contract has as its major purpose serological diagnosis for the presence or absence of mouse ectromelia, LCM, polyoma, M. hepatitis, M. adenovirus, PVM, Sendai, Theiler's (GDVII), K, Rheo 3, SV5, Toolan's H-1. The contractor also carries out ancillary research concerned with the effects of viruses, such as vaccinia and polyoma, on the growth of tumors; interaction of polyoma virus with tumors; antibody-producing capacity of tumor-bearing mice and the effect of the route of injection of antibody levels; and a comparison of the sensitivity of caesarian-derived and other mice to polyoma virus. This activity furnishes information on the effects of "Passenger" agents on the growth of tumors used in the screen and on their effects on drug action. During a contract period of one year, this laboratory performs approximately 20,000 rodent serum and 2,000 tumor fragment diagnostic tests. The contractor also prepares large quantities (40,000 ml. yearly) or vaccinia virus used for immunizing mice against ectromelia.

NEW YORK UNIVERSITY (PH43-67-1174)

The purpose of this contract is to provide data retrieval support for experimental chemotherapy of selected patients with brain tumors in accordance with Brain Tumor Chemotherapy Study Protocol No. 6601, so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups, randomly selected.

NEW YORK UNIVERSITY (PH43-67-1336)

The major goals of this project are to test and evaluate methods of rapid, accurate data acquisition. New techniques of source data automation, including some direct entry by medical staff into a remote time-shared computer, are being compared with more conventional approaches to data capture. Comprehensive methods of data quality control for research protocols are also being devised including programmed data checks by computer.

OHIO STATE UNIVERSITY (PH43-67-1175)

The purpose of this contract is to provide data retrieval support for experimental chemotherapy of selected patients with brain tumors in accordance with Brain Tumor Chemotherapy Study Protocol No. 6601, so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups, randomly selected.

PARKE DAVIS AND COMPANY (SA-43-ph-4320)

This service preparative laboratory was established primarily for the resynthesis of compounds for experimental tumor studies. In general, these are compounds which have shown presumptive activity in one of the screens but for which sufficient material is not available for the completion of definitive testing. Five to twenty-five grams are normally prepared. About 35% of the effort is devoted to the preparation of larger quantities of compound for preclinical toxicology and clinical trial; quantities of these clinical compounds have been as high as 60 kilograms. The flexibility of the contractor in being able to provide either laboratory or pilot-plant quantities of compounds has provided the NCI with ready access to such materials. Drug formulations have also been carried out at the request of the NCI staff.

PERKIN-ELMER CORPORATION (PH43-66-1159)

This was a feasibility study for the development of an automated scanning device to read autoradiographs from blood, bone marrow, and other tissues. A scanner was developed which is now in use as part of an intramural effort in automated scanning and pattern recognition.

PFIZER, CHARLES AND COMPANY, INC. (PH43-67-687)

This contract provides for the operation of a pharmaceutical pilot plant for dosage form development and production. Functions include: preparation of parenteral and oral dosage forms of antitumor compounds; development of dosage forms for new drugs; establishment of testing systems for identity, purity, sterility, and stability of the final product; and preparation of records and labels for each lot of material produced.

Those compounds originating from the contractor remain at the site for storage and distribution under the direction of the NCI chemotherapy staff.

This contract was assigned 61 development and formulation projects during calendar year 1967. The production achieved during that year amounted 60,000 capsules, 88,578 vials, and 5,200 tablets.

PFIZER, CHARLES AND COMPANY, INC. (PH43-68-45)

This contract represents a comprehensive drug development program for screening, isolation, characterization, synthesis and toxicologic and pharmacologic evaluation of antitumor agents, primarily fermentation and plant products. The scope also includes the maintenance of an inbred mouse colony for producing animals needed for the various screening assays. Screening work has been concentrated on the two-tumor screen employing L1210 leukemia and the Walker 256 intramuscular tumor systems. These systems are used to follow isolation work and testing of antibiotic broths, plant extracts and synthetic chemicals. Other systems are also used for special purposes.

This laboratory effort includes the toxicologic evaluation of compounds developed under contract or submitted for evaluation by the Laboratory of Chemical Pharmacology. The studies are directed toward describing the qualitative and quantitative toxicity of potential clinical agents. The toxicity is characterized as to its predictability, reversibility, and relationship to cell cycle kinetics. The maximum tolerated dose serves as the basis for the initial clinical dose level. The examination of antidotes and countermeasures against the toxicity of selected oncolytic agents also constitutes an important function of the subject contract. Additionally, efforts formerly devoted to the development and evaluation of new screening methods are now being expended in the preparation of radiolabeled drugs and the use of such drugs for blood level, excretion, distribution and other related studies in preparation for the more extensive clinical pharmacology now being conducted by the several Clinical Cooperative groups.

PICO LABORATORIES, INC. (PH43-67-1167)

The primary objective of this contract is to evaluate the chronic toxicity to laboratory animals of cancer chemotherapeutic agents and combinations of agents as requested by NCI staff. Since this organization was new to NIH when the contract was initiated in June, 1967, and had been in business only since June, 1966, the scope and size of the contract were limited to allow demonstration of performance capability.

The initial research was a toxicologic investigation of the oral administration of o,p'-DDD to rats and beagle dogs for twenty-eight days. This work has been performed in a satisfactory manner, and the analytic capabilities of the laboratory were used to additional advantage in connection with a cooperative study, carried out in conjunction with Hazleton Laboratories, on the fetal effects of o,p'-DDD administered to pregnant bitches.

PITTSBURGH, UNIVERSITY OF (PH43-67-1186)

The main objective of this contract is the structural determination of materials of interest to the program by X-ray crystallographic techniques. The project is limited to the study of materials whose structures are otherwise difficult to establish.

PUBLIC HEALTH SERVICE DIVISION OF HOSPITALS (TRANSFER OF FUNDS)
(Baltimore, Maryland; Boston, Massachusetts; & New Orleans, Louisiana)

This transfer of funds involves the support of three Public Health Service Hospitals for the evaluation of antitumor agents clinically. A major portion of the effort involves the establishment and operation of clinical pharmacology activity at the Baltimore Public Health Service Hospital.

RAWLEY FARMS (PH43-68-625 and PH43-68-703)

These procurement contracts supply 239,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice for screening tests. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

RESEARCH FOUNDATION OF THE STATE UNIVERSITY OF NEW YORK (PH43-66-973)

This was a search for and study of records of long-term survivors of acute leukemia. The complete records of long-term survivors (over 5 years) with this disease were obtained. Approximately 150 such individuals were identified and analyzed. A report is in preparation. The contract was terminated in FY 1968.

RESEARCH TRIANGLE INSTITUTE (SA-43-ph-4322)

The purpose of this contract is to fractionate confirmed active plant extracts in an attempt to isolate in a pure state and identify the active compounds. Plant material used for this work is obtained, for the most part, through the U. S. Department of Agriculture.

The contractor has many plants which are or have been in some stage of fractionation, several of which have been dropped for lack of reproducible activity or because they were found to contain compounds already known to the program. Several active agents have been isolated and are currently being subjected to additional evaluation. One material, the soluble sodium derivative of camptothecin, has been selected as a candidate for preclinical toxicology and clinical trial and is being prepared in quantity under another contract.

RIKER LABORATORIES (PH43-66-904)

This service preparative contract provides for the resynthesis of a wide variety of compounds for both clinical and experimental therapeutic purposes. Clinical materials represent about 30% of the total effort. The materials prepared under this contract are those which cannot be obtained from the original supplier in the necessary quantities. This laboratory has access to large-scale facilities and is, therefore, able to prepare kilogram quantities of agents when needed. Because of the need for plant facilities by the contractor, this contract will, at their request, terminate early in FY 1969.

ROMA KENNELS (PH43-68-3)

This procurement contract provides for the supply of 120 beagles for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the contractor.

SCHMIDT, A. R., COMPANY (PH43-66-86)

This contract has as its objectives the development of germ-free foundation colonies of inbred rodents required for program studies. Pedigreed animals are derived via hysterotomy and foster suckled in germ-free isolators. Selected pedigreed offspring are artificially contaminated with pure cultures of organisms and are developed as pedigreed expansion colonies in jacket isolators. Offspring from this second stage will be issued to secondary genetic centers, who in turn will produce breeding animals for large-scale production colonies. Classic methods for the maintenance of the animals are followed with respect to environmental controls and microbiological monitoring.

SCHMIDT, A. R., COMPANY (PH43-66-484)

This contract provides for the maintenance of a center to produce random-bred albino rats as breeding animals for large-scale production colonies. Pregnant rats are obtained from an in-house production colony, derived by means of the hysterotomy-isolator techniques and established in germ-free plastic isolators. Offspring of these rats are removed to other isolators wherein they are artificially contaminated with known microflora, and established as a production colony. Offspring of these rats, in turn, are shipped to the large-scale production colonies. The latter supply test animals for compound evaluation studies.

SCHMIDT, A. R., COMPANY (PH43-66-901)

The original objective of this contract was to develop a colony of Syrian hamsters free of the commonly occurring pathogens. Due to the lack of success in maintaining germ-free hamsters beyond the 7th or 8th day of life, emphasis is being placed upon the development of "clean" hamsters which are free of ecto- and endoparasites, and bacterial and known viral contaminants. Offspring serve as breeding animals in large-scale production colonies under controlled environmental factors. Germ-free hamster studies will be continued concurrently but at a minor level unless a "break-through" takes place.

SCHMIDT, A. R., COMPANY (PH43-67-94)

This procurement contract provided about 52,000 Fischer 344 rats for drug evaluation studies. Originally, breeding animals were derived by the hysterotomy-isolator techniques in order to reduce disease levels and eliminate diseases of major importance.

This contract terminated December 31, 1967.

SCHMIDT, A. R., COMPANY (PH43-67-110)

This contract provides for the maintenance of a Genetic-Production Center. Mice not required for the continuity of foundation stocks are supplied to large-scale production colonies for breeding purposes and to laboratories performing studies under contract. Periodically small colonies of specific strains (DBA/2, C57BL/6, and BALB/c) are activated in order to furnish limited numbers of mice for short-term studies. Strains of mice not required for current studies are maintained at repository levels.

SCHMIDT, A. R., COMPANY (PH43-68-21)

This contract furnishes approximately 96,500 random-bred rats for drug evaluation studies. Breeding animals with defined microflora are provided by the Government. The parents of the breeding rats are produced by means of the hysterotomy-isolator techniques.

SCHMIDT, A. R., COMPANY (PH43-68-682)

This procurement contract provides 130,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for screening tests. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

SCRIPPS CLINIC AND RESEARCH FOUNDATION (PH43-65-14)

The purpose of this contract is to study, at the molecular level, the mode of action of folic acid antagonists used in the chemotherapy of cancer. Highly purified dihydrofolic reductase has been prepared from chicken liver, L1210 cells and from human leukemic cells. Isoenzymes are also being sought. The purified enzymes are being characterized, the kinetics of the binding of analogs to them determined, and studies made of new drugs which will represent structural variations of the folate molecule.

Dihydrofolic reductase is considered to be the specific target of methotrexate. Thus, knowledge of whether the enzyme is identical in normal and malignant tissues, mouse and man, in sensitive and resistant tumors, whether there are isoenzymes, and the nature of the active site should be useful in achieving the maximum therapeutic effect with methotrexate itself and in the possible design of desirable new drugs.

SIMONSEN LABORATORIES, INC. (PH43-65-55)

The contractor maintains a genetic trust stock colony of six inbred strains of rodents. Small quantities of animals from the colony are made available for tumor transplantation and the majority are furnished for large-scale production propagation.

SIMONSEN LABORATORIES, INC. (PH43-68-32)

This procurement contract is designed to furnish yearly 26,000 ten-week-old CDF_1 mice (BALB/c♀ x DBA/2♂) free of pseudomonas for CCNSC contract studies.

SIMONSEN LABORATORIES, INC. (PH43-68-32) con't.

Breeding animals are furnished by the Government. The mice are used mainly for spleen colony studies. The mice are held on chlorine-water for about four weeks beyond the normal shipping time

SIMONSEN LABORATORIES, INC. (PH43-68-698)

This procurement contract provides 156,000 BDF1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for screening tests. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

SOUTHERN ANIMAL FARMS (PH43-68-31)

This procurement contract is designed to furnish yearly 52,000 six-week-old CDF_1 (BALB/c♀ x DBA/2♂) hybrid mice free of pseudomonas for CCNSC contract studies. Breeding animals originate from genetic centers. The mice are offered chlorinated water in order to eliminate or control the incidence of pseudomonas.

SOUTHERN ANIMAL FARMS (PH43-68-701)

This procurement contract provides 104,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for screening tests. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

SOUTHERN RESEARCH INSTITUTE (PH43-63-68)

This contract employs three virus systems for in vivo testing (Friend virus, Rous sarcoma virus and Salivary virus).

In addition, vaccinia virus, polyoma virus and influenza are available for secondary testing. A total of 68 compounds were tested in the in vivo Friend Leukemia virus system, 48 in the Rous sarcoma virus and 56 in the Salivary gland system. A total of 17 compounds were tested in in vivo vaccinia system by special request from either the supplier or project officer. The salivary gland virus is a murine virus similar to the human cytomegalovirus which is of clinical interest since patients treated with immunosuppressive agents shed this virus. At present the cytomegalovirus system is an in vitro test.

This contract will terminate in June 1968. Further effort will be expended in developing a suitable cytomegalovirus system under contract PH43-65-594 at Southern Research Institute.

SOUTHERN RESEARCH INSTITUTE (PH43-64-51)

Southern Research Institute is one of the basic synthesis contractors for the CCNSC. The chemistry program is oriented to the biological and biochemical rationales developed in other programs, and is integrated with the screening, therapeutic, and biochemical activities at Southern Research Institute. The areas of investigation include: purine antimetabolites, including 9-substituted-9H-purines and ring analogs of purines; folic acid antagonists, directed

SOUTHERN RESEARCH INSTITUTE (PH43-64-51) con't.

towards the preparation of "simpler" derivatives and variants of the methotrexate and aminopterin structures; imidazole antimetabolites and analogs of imidazoles; nucleotides; _N_-nitrosourea analogs; derivatives of vitamin B_6; and aryl and heterocyclic triazenes.

SOUTHERN RESEARCH INSTITUTE (PH43-65-594)

This project represents a broad, integrated effort in experimental chemotherapy, ranging from routine screening to methods development and basic research studies. Primary _in vivo_ screening is conducted at a rate of 12,000 - 14,000 tests per year. Schedule and route dependency studies and associated toxicity tests account for approximately 15,000 additional tests. Thus, the actual work load in rodent-tumor systems approximates 30,000 tests per year. Detailed evaluation of new and old drugs with respect to schedule and route dependency includes studies of therapeutic indices, dose-response relationships, structure-activity relationships, etc. Combination chemotherapy and chemotherapy of drug resistant tumors is carried out in order to provide leads to more effective therapies and to provide information on mechanisms of drug action and resistance.

A major objective of this project is the design and development of new model systems for the evaluation of agents as potential antitumor drugs. Basic and developmental research associated with this effort include studies of the kinetics of leukemic and solid tumor cell proliferation and the influence of therapy thereon. Such cell kill kinetic studies are conducted _in vivo_ to attempt to describe conditions for the cure of transplantable early, established, or metastatic tumors of diverse histological types and varied cells of origin as well as first generation transplants of spontaneous tumors and advanced stages of spontaneous tumor growth. Cell kill kinetic studies _in vitro_ are conducted against proliferating and non-proliferating tumor cells in order to provide information relative to the degree of cell cycle stage specificity of inhibition by an active agent. Additional cell culture work involves attempts to establish synchronized cultures of mammalian tumor cells. Tritiated thymidine studies are conducted to determine the generation time of tumor cells and the portion of the life cycle that the cell is engaged in DNA synthesis. A small number of standard cytotoxicity tests _in vitro_ are conducted with new agents synthesized under contract PH43-64-51 and in conjunction with biochemical and microbiological studies under contracts PH43-65-654 and PH43-66-29 at this Institute.

Microbiological testing emphasizes the development of assays for the detection and determination of antitumor agents in body fluids and tissues, screening of selected compounds for differential inhibition of an asparagine-requiring mutant of _S. bovis_, searches for therapeutically potentiating drug combinations, evaluation of new agents to relate them to known agents, the use of bacterial cultures as models for studying the kinetics of mammalian tumor cell response to antitumor agents, and screening of agents for antimicrobial activity _in vitro_ and _in vivo_ for possible control of fulminating bacterial and fungal disease in drug-treated tumor patients.

SOUTHERN RESEARCH INSTITUTE (PH43-65-594) con't.

A modest effort is devoted to tumor immunology studies with transplantable murine leukemia. With the expected termination of contract PH43-63-68 with this Institute, it is expected that part of the latter project will be continued under contract PH43-65-594: the evaluation of antiviral and antitumor drugs *in vitro* against human cytomegalovirus (CMV).

SOUTHERN RESEARCH INSTITUTE (PH43-65-654)

This contract is concerned with the development of chemical and/or microbiological methods for measuring antineoplastic agents in biological fluids and tissues. Such methods will then be applied to the study of the physiological disposition of these agents in normal and tumor-bearing laboratory animals and, in collaboration with clinical investigators, in man. Plasma and tissue drug and metabolite concentrations will be correlated with antitumor activity and host toxicity.

SOUTHERN RESEARCH INSTITUTE (PH43-66-29)

The major objective of this contract is to develop basic knowledge that might be used in the design of new agents or in the more effective use of known agents. This objective is pursued through study of biochemical aspects of cancer chemotherapy of problems relating to: (a) sites of action and the metabolism of compounds with antitumor activity; (b) the biochemical basis for resistance to important antitumor agents; and (c) study of the dynamics of cell growth and the effects of antitumor agents thereon.

The mechanism of action of alkylating agents continues to be studied in sensitive and resistant plasmacytomas bilaterally implanted in hamsters. Purine analogs are studied in a spectrum of resistant H. Ep. #2 lines, each of which lacks enzymes important in purine nucleic acid synthesis, and by the determination of their effect *in vivo* on the incorporation into nucleic acids of labeled intermediates known to enter the synthetic pathway at different points. Because *Tetrahymena pyriformis* lacks *de novo* synthetic ability for purine and pyrimidine bases, analog incorporation into its nucleic acids was studied.

The kinetics of cell growth were investigated, by the use of tritiated thymidine incorporation in a variety of tumors, in studies related to the determination of the rates of growth of cells, analysis of the cell cycle, the effects of drugs on the cell cycle, and the utilization of such knowledge in making more effective use of chemotherapeutic agents.

SOUTH SHORE ANALYTICAL AND RESEARCH LABORATORY (PH43-64-932)

The routine toxicologic evaluation of 9 to 32 compounds a year is pursued under this service-type contract. Laboratory facilities and personnel are adequate for following leads developed during preliminary experiments.

The studies are directed toward defining the qualitative and quantitative toxicity of potential clinical cancer chemotherapeutic compounds. The toxicity is characterized as to predictability, reversibility, relationship to cell cycle kinetics and temporal relationship to termination of dosing.

SQUIBB, E. R., AND SONS (PH43-65-1056)

This contract provides a facility for (a) fermentation and isolation studies on confirmed actives from Squibb's antitumor antibiotic program, (b) large-scale preparation of fermentation products for toxicology and clinical trial when original supplier is unable to provide the quantity needed, (c) large-scale preparation of materials needed for the NCI program, which were previously supplied in smaller quantities under the contract, (d) preparation and quality control testing of dosage forms of clinical materials produced under the contract, and (e) custom preparation of fermentation products, or other natural products, not available elsewhere and other materials as requested by the Project Officer.

The contract has supplied Restrictocin for clinical use, Rubiflavin, and a selection of fatty acid analogs of Septacidin. The last two will be submitted as clinical candidates. Custom fermentation studies have been conducted on bostrycoidin, azacolutin and duramycin.

Developmental studies were completed and clinical material prepared both of Saramycetin and L-Asparaginase. A major portion of this year's effort has been directed toward the latter product. Recovery of pilot plant quantities of both camptothecin and thalicarpine have been accomplished from the natural source materials, supplied by the NCI.

STANFORD RESEARCH INSTITUTE (PH43-64-500)

Stanford Research Institute is one of the three major contractors devoted to the synthesis of new chemical structures. The research program encompasses such areas as nucleosides, antimetabolites, antibiotics, alkaloids, and other areas as may be requested by NCI.

The completion of the preparation of "homofolic acid" has opened the path for the synthesis of "homoaminopterin" and related compounds. Work is also in progress on other analogs of tetrahydrofolic acid, particularly compounds having high activity as inhibitors of dihydrofolic reductase and/or thymidylic synthetase.

Exploitation of the leads in the Vinca alkaloid field is being pursued by synthesis of new indole alkaloids. Compounds related to the known indole alkaloids ellipticine and olivacine are also under investigation.

Special attention is being directed to the study of the chemistry of the antibiotic Daunomycin in order to develop agents of greater specificity at the molecular level. A modest effort is carried out in the polyhydroxyanthranquinone-type compounds. This structure occurs in Daunomycin and many other antibiotics. Such materials often demonstrate a wide variety of antitumor activity in both animals and man. Associated with hydroxylated quinones are sugar moieties, which are also unique and merit investigation. These trideoxyamino sugars are also under investigation.

STANDARD RESEARCH INSTITUTE (PH43-64-500) con't.

Over 20% of this contract is concerned with quality control studies on compounds prepared for preclinical toxicology and clinical trial. The contractor evaluates bulk chemicals and formulated drugs as to their identity, assay, and purity, to provide assurance of high quality of the drug; as to material and dosage forms for the clinical trials; and to provide some of the necessary analytical data for the Food and Drug Administration.

This area of work provides elemental analysis, as well as the more sophisticated techniques such as chromatography (paper, thin layer, and gas), absorption spectroscopy (ultraviolet, visible, and infrared), nuclear magnetic resonance, and other physical-chemical procedures appropriate for the situation. In some cases methods of assay must be developed for new structures in order to maintain adequate surveillance on the quality of all drugs used in clinical trials.

STANFORD RESEARCH INSTITUTE (PH43-65-575)

The purpose of this contract is to study the mechanism of action of nucleoside analogs in mammalian tissues. The first step in such studies is to determine the antitumor activity of prospective agents in a battery of ascitic tumors of different metabolic patterns. Clues as to area of activity so obtained are followed quantitatively with suitably chosen labeled metabolites.

The effects of the anabolic and catabolic enzymes which are concerned in the therapeutic use of agents of interest are studied in both animal and human tissue.

Because of budgetary restrictions, this contract will terminate early in FY 1969.

STANFORD RESEARCH INSTITUTE (PH43-67-1143)

This contract is concerned with the chronic toxicologic evaluation of antineoplastic drugs which are used for extended maintenance and in the treatment of psoriasis, collagen diseases and for immunologic suppression.

Answers are being sought to the following questions: (1) will the bone marrow continue to demonstrate reversibility of toxicity during a long course of serial insults or will the end result be one of gradual and complete myelofibrosis; (2) what is the tolerance of the major organ systems to prolonged drug therapy and can function impairment be reversed by withdrawal of drug; and (3) what unusual toxicologic phenomenon might be encountered during the long term administration of a specific agent.

STARKS ASSOCIATES, INC. (PH43-62-484)

This service preparative contract supplies the CCNSC with compounds required for drug evaluation studies, with 40% of the effort being devoted to the production of clinical materials. Preparations vary in quantity from 5 grams to multi-kilograms. Many of the compounds are potential folic acid antagonists and irreversible inhibitors.

TACONIC FARMS, INC. (PH43-68-695)

This procurement contract supplies 130,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for screening tests. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

TEXAS INBRED MICE COMPANY (PH43-68-88)

This contract supplies a variety of inbred and hybrid rodents for tumor transplantation; for hybrid production (at Texas Inbred and other animal production contractors); and for compound evaluation studies. Animals are supplied from a colony of 25 strains of rodents. The contractor also serves as a trust stock repository for the inbred strains of rodents.

TEXAS INBRED MICE COMPANY (PH43-68-696)

This procurement contract supplies 78,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for screening tests. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

TEXAS INBRED MICE COMPANY (PH43-68-962)

This procurement contract is designed to furnish yearly 78,000 CDF_1 (BALB/c♀ x DBA/2♂) six week old hybrid mice free of pseudomonas for CCNSC contract studies. Breeding animals originate from genetic centers. The mice are offered chlorinated water in order to eliminate or control the incidence of pseudomonas.

TEXAS, UNIVERSITY OF (PH43-66-1156)

The purpose of this contract is to develop a preclinical and clinical pharmacology facility at M. D. Anderson Hospital and Tumor Institute. Methods for the determination of anticancer drugs and their metabolites will be developed and then applied to studies with laboratory animals and patients with neoplastic diseases. Plasma and tissue drug and metabolite concentrations will be correlated with antitumor activity and host toxicity.

TRAVENOL LABORATORIES, INC. (PH43-67-1403)

The objective of this contract is the development and procurement of plastic containers for frozen platelet concentrates. These containers are designed to serve for the collection of the concentrates, storage and freezing, shipping, thawing and administration to the recipient. There is an intensive search for better plastics and improvements in container design.

This contract will terminate June 28, 1968.

TULANE UNIVERSITY (PH43-65-593)

This contract provides for the establishment of a foundation colony of caesarian-derived inbred rats (strains M520, OM/N, Fischer 344, Buffalo, W/L, ALB, ACI, and other inbred strains as required). Its objectives are to improve the quality and maintain the genetic characteristics of the strains for program activities.

TYCO LABORATORIES, INC. (PH43-67-723)

This contract, which started the latter part of FY 1967, provided for the preparation of extracts from a number of pure cultures of algae in sufficient quantities to provide two specified samples from each culture. These samples (ethanol:water extracts) are screened for *in vivo* activity in animal tumor systems. It is hoped that algae will provide a new source of unique antitumor agents.

UPJOHN COMPANY (PH43-62-168)

This contract has for its objectives the isolation, purification, characterization and production in quantity of potentially useful antitumor agents, from fungus and actinomycete fermentation beers.

The Upjohn Company has played a leading role in the exploitation of cell culture methods, both for assays on fractionation samples and for primary screening. The methods used for primary screening have been under constant review, development, and revision, thus providing a dynamic operation. The present screen, aimed at the detection of new types of antitumor leads, consists of a search for drugs with specific antimetabolite activity, followed by the determination of activity in the L1210 and Walker 256 tumor systems.

This contract has provided six antibiotics (Pactamycin, Porfiromycin, Sparsomycin, Tubercidin, Nogalamycin, and Streptozotocin) for clinical trial prior to this year. Seven new compounds were submitted to NCI for evaluation during 1967. Upon request, the contractor has undertaken special clinical production assignments. One of these materials, trifluorothymidine (F_3TDR), is now in clinical trial.

Also included in this contract are studies on the mechanism of action and physiological disposition of clinical drugs developed within the contract program. These studies are carried out in collaboration with the clinical investigators studying the specific drugs.

UTAH, UNIVERSITY OF (PH43-64-925)

This contract provided for the establishment of a foundation colony of inbred germ-free mice (strains DBA/2, C57BL/6, BALB/cAn, C3H/He, A/He, and AKR/Lw) in order to improve the quality and maintain the genetic characteristics of inbred mouse strains used in program activities.

This contract will terminate June 28, 1968.

UTAH, UNIVERSITY OF (PH43-65-1041)

This contract is for the synthesis of nucleoside analogs and derivatives, including nucleoside antibiotics, for screening as antitumor agents. The compounds selected for synthesis are based upon biological and biochemical rationale. The areas of investigation include: (1) analogs of the antibiotic tubercidin and other nucleoside antibiotics, (2) imidazo[1,2-*c*] pyrimidine and imidazo[1,2-*a*]-s-triazine nucleosides, (3) imidazole and benzimidazole nucleosides, (4) 2- and 8-substituted purine nucleosides, (5) pyrazolo[3,4-*d*] pyrimidine nucleosides, and (6) 9-(2-deoxypyranosyl) purines and related derivatives. This contractor provides these difficult materials in quantities sufficient for *in vivo* antitumor evaluation by a method of synthesis developed by the principal investigator.

VETERANS ADMINISTRATION (TRANSFER OF FUNDS)

Five cooperative clinical cancer chemotherapy research groups have received support for over 10 years through this annually renewable research agreement between the National Cancer Institute and the Veterans Administration. These groups, the VA Lung Cancer Group, the VA Cancer Chemotherapy Study Group, the VA Surgical Adjuvant Group, the Pacific VA Cancer Chemotherapy Group, and the VA Urology Group, comprise over 150 investigators in 56 VA hospitals.

Major redefinition of the goals and structure of this program is now in progress and will be reflected in the FY 1969 agreement.

VINEMONT ANIMAL FARMS (PH43-68-655)

This contract furnishes 28,000 random-bred rats for drug evaluation studies. Breeding animals with defined microflora are provided by the Government. The parents of the breeding rats are produced by means of the hysterotomy-isolator techniques.

WAKE FOREST UNIVERSITY (PH43-68-950)

The purpose of this contract is to provide data retrieval support for experimental chemotherapy of selected patients with brain tumors in accordance with Brain Tumor Chemotherapy Study Protocol No. 6601, so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups, randomly selected.

WALTER REED GENERAL HOSPITAL (TRANSFER OF FUNDS)

The clinical cancer chemotherapy program at Walter Reed General Hospital participates in the studies of Acute Leukemia Group B and receives support through an annually renewable research agreement between the National Cancer Institute and Walter Reed Army Medical Center. This agreement was initiated ten years ago. The Walter Reed unit studies an average of 65 patients a year under group and independent protocols, the majority being patients with the acute and chronic leukemias.

WEIZMANN INSTITUTE OF SCIENCE (PL480 - AGREEMENT NO. 635125)

The objective of this contract is to seek selectivity in cancer therapy through modification of the physical properties of anticancer agents by various oligomers and polymers.

To achieve this objective studies are underway to elucidate the factors which determine the interaction of cells with oligomers and polymers of many types, starting with polypeptides which are homopolymers, copolymers and those containing tritium and fluorescent-labeled polypeptides. With these materials knowledge will be gained about:

a. The relationship between cell penetration and chain length, shape, composition, charge, etc. Labeled compounds will serve to locate within the cell compounds which do penetrate.

b. The interaction between these materials and cell surfaces and macromolecular cell constituents. Since some of the synthetic polypeptides act as synthetic antigens, the relationships between synthetic material and antibody production will be explored.

c. The pharmacological action of the polymers on normal and malignant cells. This will include studies of cellular, tissue and organ toxicity, sites of localization, blood levels, excretion and metabolic alteration. Possible differences in pharmacological response of normal and malignant cells will be explored. Studies on the alteration of cellular structure, mitotic patterns, and growth differentiation will be included.

WISCONSIN ALUMNI RESEARCH FOUNDATION (PH43-66-494)

This contract provides for the *in vivo* screening of synthetics and natural products at the level of approximately 30,000 tests per annum according to CCNSC protocols. When necessary or upon request protocol toxicity tests are performed. During FY 1968 routine screening has been conducted in the murine Lymphoid Leukemia L1210 (3LE) and the rat Walker 256 intramuscular (5WM) tumor system. Special studies are being conducted on a small scale with the P388 Lymphocytic Leukemia mouse tumor and upon request specific alkylating agents are tested against the Walker 256 tumor resistant to Cytoxan (5WC). Compounds of potential interest for clinical trial are submitted for special treatment schedule studies. Methodology and/or research and development is devoted to specific problems that arise in the routine screening of agents.

WISCONSIN ALUMNI RESEARCH FOUNDATION (PH43-67-747)

The purpose of this contract is to prepare a 50% aqueous ethanol extract of plant materials at the rate of approximately 2,500 extracts per year. A semi-quantitative test for alkaloids and tannins is run on plant materials of sufficient interest to be considered for fractionation. The bulk of the contractor's plants are obtained through a transfer of funds agreement between the NCI and the U. S. Department of Agriculture.

WISCONSIN, UNIVERSITY OF (PH43-64-551)

This contract provides for fractionating and isolating the active agents from confirmed active plant extracts prepared by other contractors, primarily WARF and the University of Costa Rica. A number of plants are currently under fractionation with several nearing the final stages of purification. One material stemming from this contract was previously selected as a candidate for preclinical toxicology and clinical trial.

WISCONSIN, UNIVERSITY OF (PH43-67-1126)

This contract provides a rapid assay, utilizing cell culture techniques, as an aid in the purification of potential antitumor agents from plant materials. The materials tested are the fractionation products, prepared under contract number PH43-64-551, to isolate the active agents from materials having established antitumor activity in the CCNSC *in vivo* screen. The principal methods used are an agar diffusion system and bioautography of paper chromatograms. This technique requires only small amounts of material, which expedites the isolation work to make agents available for evaluation more rapidly.

YALE UNIVERSITY SCHOOL OF MEDICINE (PH43-67-1193)

The objective of this contract is to provide *in vitro* screening systems to aid the selection and design of promising anticancer agents of the antifolate type through assessment of the effects of agents on two enzymes from human sources: (1) dihydrofolate reductase, the target of antifolate chemotherapy, and (2) liver aldehyde oxidase which catabolizes some folate antagonists by hydroxylation.

ZUCCA HAMSTERY (PH43-68-27)

This contract provides 26,000 hamsters produced in a controlled environment. Breeding animals raised in isolators are furnished by the Government. The parents of the breeding animals are produced under environmental controls that serve to restrict the profile of intestinal microflora and viral agents. The hamsters produced under the terms of this contract are furnished for compound evaluation studies.

ZUCCA HAMSTERY (PH43-68-637)

This procurement contract supplies 26,000 random-bred Syrian hamsters annually, which are used for compound evaluation studies. Breeding animals are supplied by the contractor.

ANNUAL REPORT

CANCER THERAPY EVALUATION BRANCH

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

July 1, 1967 - June 30, 1968

Introduction

The Cancer Therapy Evaluation Branch has, during the past year, provided administrative and scientific supervision and coordination for twelve cooperative clinical chemotherapy groups, five task forces, a Special Review Committee, and special extramural programs investigating the areas of protective environment and platelet transfusion.

In addition, the Branch has recently been assigned the responsibility for the Clinical Drug Unit which collates and files all data on investigational new drugs with the Food and Drug Administration and processes all drug requests from cooperative group and independent investigators.

As a consequence of the continuing scientific reappraisal and redefinition of the Chemotherapy drug development program by NCI staff, the Chemotherapy Advisory Committee, and the National Advisory Cancer Council, as well as a thorough reconsideration of all NCI cooperative studies, major reorganization of the Chemotherapy clinical trials program is in progress. As one facet of this reorganization the cooperative groups and the Special Review Committee for which this Branch has been responsible will be transferred back to Extramural Activities where organizational responsibility for all grant-supported cooperative clinical studies will be consolidated.

In the meantime, the Chemotherapy task forces are undergoing major organizational changes. The responsibilities of executive secretary and other management aspects of certain of these task forces, formerly vested in this Branch, are in the process of being transferred under grant support to task force chairman's institutions. This transfer has been accomplished for the Polycythemia Vera Study Group and Lymphoma Task Force, while the grant for this purpose from the Chronic Leukemia-Myeloma Task Force is now under review.

Cooperative Cancer Chemotherapy Groups

Ten of the twelve cooperative groups supervised by the Branch during the past two years will be transferred to Extramural Activities. Two groups, the VA Lung Cancer Group and the VA Surgical Adjuvant Group, will be retained in the Chemotherapy program as a major resource of the most common malignancies of man.

During the past year the support of the data-retrieval and analysis activities of the cooperative groups has been transferred from contract to grant funds. This change in method of funding was carried out to enhance the relationship between these essential group components and to facilitate the simultaneous review of these group functions by the same review body.

Although the five Veterans Administration groups are supported by a transfer of funds to the Veterans Administration under an annually renewable research agreement, the remaining seven groups are supported by research grants under a specific fund allocation: "Programmed Chemotherapy." The limitation and the additional obligations (data retrieval and analysis services) on this fund made it necessary in Fiscal Year 1968 to reduce the level of support for the seven groups an average of 20% below the approved and committed levels. As a result of this reduction of support, all of these groups have critically reviewed their activities and eliminated their least productive studies.

The Cancer Chemotherapy Collaborative Clinical Trials Review Committee has applied and is continuing to apply stringent criteria to the review of the studies, funding, and membership of these groups. This Committee has been requested by the National Advisory Cancer Council to re-examine the entirety of the collaborative clinical cancer research program in the light of modern knowledge and requirements. A meeting for this purpose is scheduled early in FY-69. Brief summaries of the organization, studies, and bibliography of these twelve cooperative groups are appended.

Special Programs

The Leukemia Center Program supported by a specific Congressional appropriation of $1.5 million, has been in effect for two years. These funds provide supportive care, platelet transfusions, domiciliary care, and travel for cancer patients, with emphasis on children with leukemia. Thirty-two investigators and institutions participate in this program, both with and without cooperative-group affiliation. Research emphasis is on the preparation, preservation, administration, and evaluation of the efficacy of platelet transfusions. A total evaluation of the first eighteen months of operation of this program is now under way, and will serve as a guide to its future administration.

The Life Island Program, in effect at an annual level of $600,000 for the past two years, supports the research at four institutions in the problems and values of major attempts to control infections in patients receiving cancer therapy. These attempts include the maintenance of sterile environments within plastic tents ("Life Islands"), the controlled or "laminar flow" of uncontaminated air within the patient's room, and the use of massive antibiotic therapy and other special techniques to reduce contamination with infectious agents.

These two research programs on the development and application of specialized techniques of supportive care offer the possibility of more effective use of currently available and newly developed anticancer agents. Control of hemorrhage (by platelet transfusions) and prevention of infection (by "Life Islands") are proving to be valuable adjuncts to modern cancer therapy.

Task Forces

The Acute Leukemia, Chronic Leukemia-Myeloma, Lymphoma, Solid Tumor, and Polycythemia Vera Task Forces are disease-oriented, scientific advisory groups, organized and supervised within this Branch. These advisory groups were organized in 1962 and thereafter, consist of preclinical and clinical experts in their respective fields, and are charged with the evaluation of current investigations and the identification of new, promising leads for future research in specific diseases.

During the past year the concept of transferring the management of certain of these task forces to the chairman's institution under grant support was developed. The management of the Polycythemia Task Force, now evolved into a functioning cooperative group, and of the Lymphoma Task Force has been transferred to their respective chairman's institutions, and a grant application for a similar arrangement for the Chronic Leukemia-Myeloma Task Force is pending review.

Brief summaries of the history, goals, and accomplishments of these task forces are appended.

The effectiveness of the "task-force" concept has led to the formation of other such groups with various goals. These do not fall within the organizational structure of the Cancer Therapy Evaluation Branch.

The Clinical Trials Task Force was organized in 1966 to accelerate the development of drugs for clinical trial and to coordinate and evaluate the studies of these drugs.

The Breast Cancer Task Force was established in Fiscal Year 1966 by specific Congressional appropriation. The annual report of this Task Force will be found within the annual report of the Scientific Director for General Laboratories and Clinics, Endocrinology Branch.

The Brain Tumor Chemotherapy Study Group, made up of neurosurgeons, has initiated a collaborative program under the auspices of NINDB and the NCI. A more complete description of this study group will be found in the annual report of the Associate Scientific Director for Experimental Therapeutics, Scientific Director for Chemotherapy.

The Choriocarcinoma Task Force was organized under the chairmanship of Dr. Roy Hertz to apply our present therapeutic knowledge to the control of choriocarcinoma in women. The description of this Task Force will be found in the annual report of the Associate Director for Extramural Activities.

Other task forces recommended by the National Advisory Cancer Council and presently in the process of organization by the Director of the National Cancer Institute, include the Lung Cancer Task Force, the Colo-Rectal Carcinoma Task Force, and the Bladder-Prostate Cancer Task Force.

It is anticipated that these task forces with their concentration of enthusiastic, highly qualified experts will provide new leads and new impetus to cancer research.

CANCER THERAPY EVALUATION BRANCH

COOPERATIVE CANCER THERAPY GROUPS

	GROUP	CHAIRMAN	INSTITUTIONS
1.	Acute Leukemia Cooperative Group B	Dr. James F. Holland	35
2.	Children's Cancer Study Group A	Dr. John Hartmann	21
3.	Central Clinical Drug Evaluation Program	Dr. Anthony Curreri	32
4.	Eastern Cooperative Oncology Group	Dr. Bruce Shnider	24
5.	Southeastern Cancer Chemotherapy Cooperative Study Group	Dr. Charles M. Huguley	12
6.	Southwest Cancer Chemotherapy Study Group	Dr. H. Grant Taylor	19
7.	Western Cooperative Cancer Chemotherapy Group	Dr. Joseph Bateman	14
	Veterans Administration Groups		
8.	VA Chemotherapy Study Group	Dr. Henry Close	13
9.	VA Lung Cancer Group	Dr. Julius Wolf	19
10.	VA Pacific Chemotherapy Group	Dr. Jack Brook	13
11.	VA Surgery Adjuvant Group	Dr. George Higgins	25
12.	VA Urologic Group	Dr. George Mellinger	20

TASK FORCES

Acute Leukemia Task Force	Dr. C. Gordon Zubrod, Chairman	NCI
	Dr. B. Ken Gray, Exec. Sec.	NCI
Chronic Leukemia-Myeloma Task Force	Dr. Charles M. Huguley, Chairman	Emory Univ.
	Dr. Edward Cahn, Exec. Sec.	NCI
Lymphoma Task Force	Dr. Emil Frei, III, Chairman	M.D. Anderson
	Dr. Jacqueline Hart, Exec. Sec.	M.D. Anderson
Solid Tumor Task Force	Dr. C. Gordon Zubrod, Chairman	NCI
	Dr. Theodore Hahn and	NCI
	Dr. Stanley Shackney, Exec. Secys.	NCI
Polycythemia Vera Study Group	Dr. Louis Wasserman, Chairman	Mt. Sinai Hosp.
	Dr. Joseph Glass, Exec. Sec.	Mt. Sinai Hosp.

ACUTE LEUKEMIA TASK FORCE

In 1962 the Acute Leukemia Task Force was formed through the efforts of its present chairman, Dr. C. Gordon Zubrod. Since its inception, the Task Force has functioned as a disease-oriented, interdisciplinary, advisory group designed to fulfill the following objectives:

(1) the control and ultimate cure of acute leukemia, and

(2) the total supportive care of the leukemic patient.

The approach to these objectives is sought through periodic forum discussions between some 50 scientists from more than 25 institutions throughout the country. Through the interdisciplinary nature of its members, catalysis and cross-fertilization of ideas and exchange of concepts have occurred, which has resulted in intensive research in platelet and granulocyte procurement, processing, storage and administration; the application of cell kinetic theory to leukemia therapy; and the investigation of new patient-protection innovations such as laminar flow and Life Island environments.

The present Acute Leukemia Task Force organization is outlined below.

I. Chemotherapy Group

  a. Pharmacology Subcommittee
  b. Burkitt Tumor Subcommittee
  c. Subhuman Subcommittee
  d. Cell Kinetics Group (sponsored group)

II. Platelet-Supportive Care Group

  a. Platelet Preservation Subcommittee
  b. Platelet Transfusion Subcommittee
  c. Patient Protection Subcommittee

III. Viral Etiology Group

The Chemotherapy Group of the Task Force has been concerned with the compilation and evaluation of data on chemical agents that have demonstrated activity against experimental or human leukemias. In addition to the identification of promising antileukemic agents, the Chemotherapy Group has explored combinations of agents in an attempt to obtain additive or synergistic therapeutic effects. The possibility of cure of human acute leukemia, based on extensive data on the cell kinetics and curability of experimental animal leukemia, is currently under intensive Task Force investigation.

Recent developments in the kinetics of normal and neoplastic cellular proliferation, cell-cycle sensitivity, and dose-schedule relationships are being considered in great detail. The Chemotherapy Group has also had a continuing interest in the study of long-term acute leukemia survivors. A registry containing information about such patients is maintained, and an epidemiological study is being conducted.

The Platelet-Supportive Care Group has been concerned with two major areas; (1) platelet and granulocyte replacement therapy, and (2) prevention and management of infectious complications. Studies on platelet cryobiology are in progress at a number of collaborating institutions, and a subcommittee has undertaken a cooperative clinical study of frozen platelets. In anticipation of the needs of the many institutions which are starting their own platelet-transfusion programs, the Platelet Group has sponsored a movie on the techniques of platelet-transfusion therapy, and a subcommittee of the Platelet Group has prepared an outline of the techniques used in platelet-transfusion therapy.

The Viral Etiology Group has functioned effectively as a forum for bringing together investigators working actively in this area with scientists from other areas of leukemia research. Ongoing work is discussed, and problems, possible leads, and clinical correlations are explored.

CHRONIC LEUKEMIA-MYELOMA TASK FORCE

The Chronic Leukemia-Myeloma Task Force traces its origin to the Chronic Leukemia and Multiple Myeloma Coordinating Committee of the Clinical Studies Panel, established in July 1964. This Committee sought to assess the then current stage of knowledge regarding the diagnosis, natural history, and therapy of the chronic leukemias and plasma cell myeloma.

It was the feeling of this Committee that the cooperative chemotherapy groups should be offered basic guidelines for the study and chemotherapy of these diseases in order to improve the evaluability and comparability of their studies. The designation of the Committee as a Task Force was made in December 1964. Dr. Charles M. Huguley, Jr. was appointed chairman in June 1965.

The Task Force is composed of one or two members representing each cooperative group and independent investigators of established reputation in its fields of interest. A Chronic Leukemia Subcommittee and a Myeloma Subcommittee consider the specific problems of these diseases and report their recommendations to the Task Force. Other subcommittees are conducting retrospective studies and investigating the possibility of establishing epidemiologic surveys in the chronic leukemias and multiple myeloma.

The Chronic Leukemia-Myeloma Task Force serves the two primary functions of coordination and education. In the first capacity, the Task Force seeks to stimulate and coordinate studies by the cooperative chemotherapy groups, to insure retrieval of the most significant data from the clinical investigations performed. Retrospective studies in the chronic leukemias are being performed to evaluate the prognostic significance of the various manifestations of these diseases and their response to therapy.

In its second function of education, the Task Force correlates and disseminates current knowledge related to its areas of interest. This includes both basic and clinical research. The morning session of each Task Force meeting is devoted to a scientific session, with papers on current research presented by Task Force members and guest investigators.

The Task Force has prepared guidelines for diagnosis and chemotherapeutic trials in chronic myelocytic leukemia, plasma cell myeloma, and chronic lymphocytic leukemia. These three sets of guidelines have been circulated to all chemotherapy cooperative groups and have received their approval. It is planned that the three guidelines will be published as a supplement to Cancer Chemotherapy Reports.

The Task Force has held special workshops presenting standardized methods of leukocyte alkaline phosphate determination and bone marrow chromosome analysis to provide the constituent institutions of the cooperative groups with uniform methods and comparable results. A central reference laboratory for protein immunophoresis of the serum and urine of myeloma patients has been set up at the suggestion of the Task Force under the supervision of Dr. John Fahey, NCI. All investigators are offered the opportunity of submitting patients' specimens to this laboratory for evaluation.

LYMPHOMA TASK FORCE

The Lymphoma Task Force was organized by Dr. Emil J. Frei, III, in 1964 at the request of the Clinical Studies Panel. The purpose of the Task Force was to review, propose, and coordinate therapeutic studies in patients with Hodgkin's disease and lymphosarcoma. The initial meeting considered the studies in progress in the cooperative chemotherapy groups and at the NCI as well as the availability and potential value of newly screened compounds. Prior to the September 1966 meeting, the membership list was revised and Dr. James Nickson, a radiologist, was asked to serve as co-chairman with Dr. Frei. This administrative addition reflected the hope of the Task Force that chemotherapists and radiotherapists working together might be able to design improved, if not curative, therapy for the malignant lymphomas. The Lymphoma Task Force is composed of radiotherapists, pathologists, and members representing all of the cooperative chemotherapy groups.

The Task Force is attempting to define those programs of intensive or combined therapy which produce the maximum complete remission rate or cure rate in malignant lymphomas, and to determine the effect of different drugs and schedules on the duration of remissions. A continued emphasis is placed on the selection of new agents for clinical trial and on the conduct of such trials.

The Lymphoma Task Force sponsored a Conference on Lymphography in January 1967 in order to stimulate wider application of this diagnostic procedure in the staging of lymphomas and the evaluation of therapies. The proceedings of this meeting were published in Cancer Chemotherapy Reports, January 1968. It is hoped that these data will be of particular interest to clinicians and diagnostic radiologists who are not presently taking advantage of this useful diagnostic tool.

A central pathology repository and review committee, the Pathology Panel of the Lymphoma Task Force, was established to implement retrospective and prospective studies by providing a correlation of precise histologic classification of the lymphomas with their clinical characteristics, response to therapy, and survival. Dr. Henry Rappaport serves as chairman of the Pathology Panel. This Panel in the past year has participated in a WHO meeting on the Burkitt tumor, sponsored a program with Japanese pathologists, and participated in a symposium on the comparative pathology of lymphomas in lower animals held at the AFIP.

Effective January 1, 1968, the office of the Task Force was transferred to the M. D. Anderson Hospital in Houston with Dr. Jacqueline Hart serving as executive secretary.

The Task Force plans to continue its present programs with particular emphasis on rapid dissemination of information regarding new agents and more effective regimens and modes of therapy. Intergroup cooperation will remain an important means of more rapid testing of hypotheses for potential control or cure of malignant lymphomas.

POLYCYTHEMIA VERA STUDY GROUP

The Polycythemia Vera Study Group, organized in 1966 under the chairmanship of Dr. Louis R. Wasserman, has, in the past year, prepared a protocol for the study of the effects of therapy on the natural history of polycythemia vera. Funding was initiated January 1, 1968 under a Public Health Service Grant. The participating institutions are:

Baltimore City Hospital
(Johns Hopkins) Md.
Brookhaven National Laboratory
Upton, Long Island, N.Y.

Dartmouth Medical College
Hanover, N.H.
Duke University Medical School
Durham, N.C.

Hektoen Institute, Ill. (University of Chicago)
Mayo Clinic, Rochester, Minn.
Montefiore Hospital, Bronx, N.Y.
Mount Sinai School of Medicine and Hospital, New York, N.Y.
National Cancer Institute, Bethesda, Md.
New England Medical Center Hospitals, Boston, Mass.
New York Hospital-Cornell Medical Center, New York, N.Y.
Ochsner Clinic-Tulane University Medical School, New Orleans, La.
Ohio State University Medical School, Columbus
Pacific Veterans Administration Cooperative Chemotherapy Group
Peter Bent Brigham Hospital Boston, Mass.
Presbyterian Hospital-University of Pennsylvania, Philadelphia
St. Elizabeth's Hospital, Boston, Mass.
University of California Medical School, Berkeley
University of Colorado Medical School, Denver
University of Oregon Medical School, Portland
University of Texas Medical Branch, Galveston
Wake Forest College, Bowman Gray School of Medicine, Winston-Salem, N.C.
Washington University School of Medicine, St. Louis, Mo.

Previously untreated patients with carefully documented polycythemia vera are being randomly allocated to one of three treatment groups: a) phlebotomy alone; b) phlebotomy plus Chlorambucil; and c) phlebotomy plus radioactive phosphorous. The protocol has been activated and patients have been entered since December 1967.

The Operations Office of the Study Group has been established at the Mount Sinai Medical School and Hospital (the chairman's institution). The Operations Office staff has been engaged in: a) designing the clinical laboratory forms that are being used for the study; b) setting up a detailed coding system for the handling of the voluminous clinical data; c) transposing the data to a form suitable for Electronic Data Processing; and d) setting up programs for the retrieval and analysis of the data.

SOLID TUMOR TASK FORCE

The Solid Tumor Task Force was organized in June 1966. Its purpose is to stimulate and evaluate intensive studies in drug therapy of the common solid tumors, specifically: breast, lung, colon, head and neck, pelvis, prostate, and genitourinary neoplasms.

The membership of this Task Force is comprised of the chairmen of the cooperative groups and other investigators active and knowledgeable in the field of solid tumor chemotherapy, with Dr. Zubrod serving as chairman.

The specific aims and activities of the Solid Tumor Task Force are to:

1. assess the present status of solid tumor chemotherapy, and compile and evaluate the available data on the clinical effectiveness of each drug in each tumor system;

2. establish criteria for the uniform evaluation of disease and response;

3. collect and evaluate cell kinetic data from various tumor systems to aid in the design of more effective drug therapies and dosage schedules;

4. present data as available on new drugs suitable for use in solid tumor chemotherapy and encourage appropriate studies, and

5. provide a forum for presentation and evaluation of data from cooperative group solid tumor studies.

Meetings to date, have been devoted to the assessment of present knowledge and methods. Common problems concerning experimental design, drug distribution, dosage schedules, case reporting, and data retrieval have come to the fore and been attacked jointly.

As a result of Task Force discussions, an Experimental Design Committee was established by the Scientific Director for Chemotherapy. This Committee serves in an advisory capacity to the cooperative groups to improve the design of current studies and to assist in the development of more efficient and productive studies in the future.

The problems of data retrieval have been extensively discussed. Data retrieval and analysis for cooperative groups previously have been achieved through statistical contracts, but such functions are now being included within the cooperative group grants to provide more responsiveness to the requirements of the collaborating investigators.

In the future, particular emphasis will be placed on:

1. attempting to improve the therapeutic ratio of available chemotherapeutic agents by altering dosage schedules in accordance with recent cell kinetic data, and

2. determining the criteria most useful for evaluating response to chemotherapy in the human solid tumors.

ACUTE LEUKEMIA COOPERATIVE GROUP B

Acute Leukemia Cooperative Group B has been in existence since 1956. Dr. James Holland has been the chairman of the Group since 1963. At the present time, this Group is composed of investigators in 35 participating institutions.

- Bowman Gray School of Medicine Winston-Salem, N.C.
- Hopital Cantonal et Policlinique, Geneva, Switzerland
- Children's Hospital of Philadelphia, Pa.
- Dartmouth Medical School, Hanover, N.H.
- Albert Einstein College of Medicine, Bronx, N.Y.
- Finsen Institute, Copenhagen, Denmark
- Hematology and Oncology Station, Zurich, Switzerland
- Institut De Recherches Sur Les Leucemies, Paris, France
- Jefferson Medical College, Philadelphia, Pa.
- Massachusetts General Hospital, Boston
- Mayo Clinic, Rochester, Minn.
- McGill University Affiliated Hospitals:
  - Montreal Children's Hospital, Canada
  - Montreal General Hospital, Canada
  - Royal Victoria Hospital, West Montreal, Canada
- Medical College of Virginia, Richmond
- Mount Sinai Hospital, New York, N.Y.
  - City Hospital at Elmhurst, N.Y.
- National Cancer Institute, Bethesda, Md.
  - USPHS Hospital, Baltimore, Md.
- New York Hospital, New York, N.Y.
  - Bronx Lebanon Hospital, N.Y.
  - South Nassau Community Hospital, Oceanside, N.Y.
- Pretoria General Hospital, South Africa
- Rhode Island Hospital, Providence
  - Veterans Administration Hospital, Providence, R.I.
- Roswell Park Memorial Institute, Buffalo, N.Y.
- Schweizerische Chemotherapiegruppe, Inselspital Bern, Switzerland
- Lemuel Shattuck, Boston, Mass.
- State University of New York (Downstate) Affiliated Hospitals:
  - Jewish Hospital of Brooklyn, N.Y.
  - Long Island Jewish Hospital, New Hyde Park, N.Y.
  - Maimonides Hospital, Brooklyn, N.Y.
- University of Miami Medical School, Florida
- Walter Reed Army Medical Center, Washington, D.C.
- West Virginia University Medical Center, Morgantown

From its inception, this Group has been interested primarily in the experimental therapeutics of acute leukemia. Over the years, however, there has been increasing interest and participation in studies of multiple myeloma, Hodgkin's disease, lymphosarcoma, the chornic leukemias, and childhood solid tumors.

The Leukemia B Group's demonstration that 6-Mercaptopurine significantly prolonged a steroid-induced remission was the first demonstration that an agent's activity could be assessed in patients without clinical evidence of disease and as such was an important contribution to subsequent studies and concepts. Extension of these concepts resulted in the demonstration of the marked superiority of intermittent parenteral Methotrexate therapy compared with the then standard daily Methotrexate maintenance schedule. Studies in progress have been designed to evaluate periodic "inducer" doses of Vincristine and Prednisone versus Daunomycin and Prednisone in 6-MP maintained remissions of acute lymphocytic leukemia in children. In addition, studies of intrathecal Arabinosyl Cytosine and evaluations of prophylactic intrathecal Methotrexate in meningeal leukemia are being carried out. A comparative study of Hydroxyurea, Hydroxyurea and Prednisone, and Daunomycin in acute myelocytic leukemia has been initiated.

A combination chemotherapy-radiotherapy study for the intensive treatment of Stage III Hodgkin's disease is nearing completion and attempts to determine whether patients with generalized disease can be cured by such a regimen. Newer agents such as Daunomycin, Procarbazine Hydrochloride and BCNU are being employed in recently initiated protocols.

Group efforts in childhood solid tumors continue to increase and new studies employ Daunomycin and BCNU in the treatment of solid tumors, Vincristine and Cyclophosphamide in the study of neuroblastoma and Vincristine, Prednisone, and Methotrexate in reticuloendotheliosis.

The statistical center for the Group, headed by Mr. Oliver Glidewell, is located with the Group headquarters in Buffalo, New York.

Recent Publications:

Jones, B., Kung, F., Hyhan, W.L., Hananian, J., Blom, J., Burgert, E.O., Jr., Mills, S.D., Treat, C., Wolman, I.J., Chevalier, L., Denton, R., Sheehe, P., Glidewell, O., and Holland, J.F.: Chemotherapy of the leukemic transformation of lymphosarcoma. J. Pediatrics 70: 442, 1967.

Burgert, E.W., Jr., and Glidewell, O., with Mills, S.D., Wolman, I.J., Kaydalp, I., Nyhan, W.L., Kung, F., Hananian, J., Sawitsky, A., Jones, B., Patterson, R.B., and Storrs, R.C.: Dactinomycin in Wilms' tumor. JAMA 199: 464, Feb. 1967.

Kyle, R.A., Carbone, P.P., Lynch, J.J., Owens, A.H., Costa, G., Silver, R.T., Cuttner, J., Harley, J.B., Leone, L.A., Shnider, B.I. (Chairman, Eastern Cooperative Oncology Group), and Holland, J.F. (Chairman, Acute Leukemia Group B): Evaluation of tryptophan mustard (NSC-62403) in patients with plasmacytic myeloma. Cancer Res. Mar. 1967.

Rege, V.B., Albala, M.M., Leone, L.A.: Six-hour intravenous infusions of 2' deoxy-5-fluorouridine (NSC-27640): Toxic effects and response rate. Cancer Chemother. Rep. 51: 81-83, April 1967.

Holland, J.F.: Daunomycin treatment in acute leukemia. *Path. Biol*. 15: 929-932, Oct. 1967.

Ellison, R.R., Carey, R.W., and Holland, J.F.: Continuous infusions of arabinosyl cytosine in patients with neoplastic disease. *Clin. Pharm. Thera*. 8: 800-809, Nov.-Dec. 1967.

Ellison, R.R., Holland, J.F., Silver, R.T., Bernard, J., and Boiron, M.: Ara-C in acute lymphocytic leukemia in adults. *Blood* (in press).

CENTRAL CLINICAL DRUG EVALUATION PROGRAM

The present Central Clinical Drug Evaluation Program group is an amalgamation of the old Western, Eastern, and Central Clinical Drug Evaluation Programs into a single group under the chairmanship of Dr. Anthony R. Curreri, University of Wisconsin. This Group was organized primarily to do Phase I and II studies of new anticancer drugs in a broad spectrum of human malignancies and to provide the correlation between results in humans and various biological screens.

Present Group membership includes investigators at the following institutions:

American Oncologic Hospital Philadelphia, Pa.
Bellevue Medical Center, New York N.Y.
Cornell University, New York, N.Y.
Duke University Medical Center, Durham, N.C.
Gunderson Clinic, LaCrosse, Wis.
Jefferson Medical College, Philadelphia, Pa.
Latter Day Saints Hospital, Salt Lake City, Utah
Mayo Clinic, Rochester, Minn.
Metropolitan Hospital, New York, N.Y.
Alton Ochsner Medical Foundation, New Orleans, La.
Ohio State University, Columbus
Presbyterian Medical Center, San Francisco, Calif.
Queens Hospital, Honolulu, Hawaii
St. Luke's Hospital, New York, N.Y.
St. Vincent's Hospital, New York, N.Y.
Temple University, Philadelphia, Pa.
Tulane University, New Orleans, La.
University of California, Los Angeles
University of Cincinnati, Ohio
University of Colorado, Denver
University of Florida, Duval Medical Center, Jacksonville
University of Illinois, Chicago
University of Kentucky, Lexington
University of Minnesota, Minneapolis
University of Oregon, Portland
University of Pennsylvania, Philadelphia
University of Rochester, N.Y.
University of Texas, South Texas Medical School, San Antonio
University of West Virginia, Morgantown
University of Wisconsin, Madison
Vanderbilt University, Nashville, Tenn.
Wadsworth Veterans Administration Hospital, Los Angeles, Calif.

Statistical services and Group headquarters operations are provided at the University of Wisconsin under the direction of Dr. Curreri and Mr. Bruce Pomeroy.

Studies conducted during the past year include evaluations of Imidazole carboxamide, F_3TDR, Testosterone enanthate, 5-Fluorouracil by 48-hour infusion, Hexamethylmelamine, Mithramycin, NSC-1026, NSC-17256E, Azotomycin, Cytosine arabinoside, and Tubercidin.

Of special interest is a recently activated Phase II study of Cytosine arabinoside in which dosage and schedule will be tailored to the kinetic behavior of the tumors of individual patients.

The Group studied and treated approximately 1000 patients in the past year. Plans for the pharmacology center have been impeded somewhat because of construction cost factors, but personnel recruitment is proceeding.

Recent Publications:

Wilson, W.L., Bisel, H.F., Krementz, E.T., Lien, R.C., and Prohaska, J.: Further clinical evaluation of 5-FUDR. *Cancer Chemother. Rep.* 51, April 1967.

Andrews, N.C., and Wilson, W.L.: Evaluation of methotrexate (NSC-740) in solid tumors. *Cancer Chemother. Rep.* 51, Dec. 1967.

Schroeder, J.M., and Weeth, J.B.: Phase II evaluation of fluorometholone (NSC-33001). *Cancer Chemother. Rep.* 51, Dec. 1967.

Mrazek, R., Andrews, N.C., Bisel, H.F., Wilson, W.L., and Hummel, R.P.: Phase II clinical study of hexestrol (NSC-9894E). *Cancer Chemother. Rep.* (in press).

Mason, J.H., Wilson, W.L., Ansfield, F.J., Rochlin, D.B., and Grage, T.: Phase I clinical study of acetophenone 2-dimethylamino-4-dihydroxy-hydrochloride (NSC-62512). *Cancer Chemother. Rep.* (in press).

Shroeder, J.M.: Oxylone, a Phase II evaluation. *Cancer Chemother. Rep.* (in press).

Andrews, N.C.: Evaluation of NSC-1026 (1-aminocyclopentane-1-carboxylic acid) in the treatment of leiomyosarcoma. *Cancer Chemother. Rep.* (in press).

Wilson, W.L., Schroeder, J.M., Bisel, H.F., Mrazek, R., and Hummel, R.P.: Phase II study of hexamethylmelamine (NSC-13875). *Cancer Chemother. Rep.* (in press).

CHILDREN'S CANCER STUDY GROUP A

The Children's Cancer Study Group A (formerly named Acute Leukemia Group A) was established in 1955. Its first chairman, Dr. Joseph Burchenal, was succeeded by Dr. M. Lois Murphy in 1960, and by Dr. John Hartmann, the present chairman, in 1965. Historically the major interest of the Group has been in acute childhood leukemia. Recently, however, increasing interest in studies of childhood solid tumors and lymphoproliferative diseases has been apparent.

The Group is composed of pediatricians and pediatric hematologists in the following participating institutions:

Babies Hospital, New York, N.Y.
Bobs Roberts Memorial Hospital, Chicago, Ill.
Cedars of Lebanon Hospital, Los Angeles, Calif.
Children's Hospital of Pittsburgh, Pa.
Children's Hospital, Denver, Colo.
Children's Hospital of Los Angeles, Calif.
Children's Orthopedic Hospital, Seattle, Wash.
Children's Memorial Hospital, Chicago, Ill.
Children's Hospital of the District of Columbia
Children's Hospital, Columbus, Ohio
Louisville Children's Hospital, Louisville, Ky.
Loyola University, Chicago, Ill.
Marquette University School of Medicine, Milwaukee, Wis.
Memorial Center, New York, N.Y.
St. Jude Hospital, Medical Building Memphis, Tenn.
University of Michigan Medical Center Ann Arbor, Mich.
University of Minnesota, Minneapolis
University of Utah, Salt Lake City
University of Rochester, N.Y.
University of Washington, Seattle
University of Wisconsin, Madison

Recent studies in patients with acute lymphocytic leukemia compare Methotrexate, Prednisone versus Vincristine, and Prednisone as reinduction regimens. Cytosine arabinoside and Daunomycin are being tested as single agents against acute childhood leukemia, and Prednisone, 6-Mercaptopurine, Vincristine, Cytosine arabinoside, and Daunomycin are being evaluated as consolidating agents.

In studies dealing with childhood solid tumors evaluations of Mitomycin C and Cytoxan in osteogenic sarcoma have been initiated and Daunomycin and Cytoxan - Vincristine combinations are being studied in neuroblastoma.

The Children's Cancer Group A has carried out significant studies in the chemotherapy of Wilms' tumor. Their evaluation of Actinomycin D and radiotherapy as adjuvants has provided the basis for the formation of a National Wilms' Tumor study which unifies chemotherapy efforts across the country in the study of this disease.

The statistical center serving the Group is located, along with the organizational headquarters, at the chairman's institution, The Children's Orthopedic Hospital in Seattle, Washington and under Dr. Edward Perrin at the University of Washington.

Recent Publications:

Borges, W.J., Nicklas, J.W., and Hamm, C.W.: Prezygotic determinants in acute leukemia. *J. Pediat*. 70: 170, Jan. 1967.

Gilchrist, G.S., and Shore, N.A.: Osteogenic imperfecta and familial leukemia. *J. Pediat*. 71: 115, Jan. 1967.

Bergman, A.B., and Schulte, C., III.: Care of the child with cancer. *Pediatrics* 40: 487-546, Mar. 1967.

Collip, D.J., Patrick, J.R., Gilchrist, G.S., Donnell, G.N., and Brubaker, C.A.: Liver glucose-6-phosphatase and carbohydrate metabolism in childhood leukemia. *Pediat. Res*. 1: 116, Mar. 1967.

Wolfe, J.A., Brubaker, C.A., Murphy, J.L., et al.: Prednisone therapy of acute childhood leukemia; prognosis and duration of response in 330 treated patients. *J. Pediat*. 70: 626-631, April 1967.

Sharp, H.L., Nesbit, M.E., D'angio, G.J., and Krivit, W.: Addition of local radiation after bone marrow remission in acute leukemia of children. *Cancer* 20: 1403-1404, Sept. 1967.

Sharp, H.L., and Krivit, W.: Renal and hepatic pathology following remission of acute leukemia due to prednisone. *Cancer* 20: 1395-1402, Sept. 1967.

Lampkin, B.C., Higgins, G.R., and Hammond, D.: Absence of neurotoxicity following massive intrathecal administration of methotrexate. *Cancer* 20: 1780-1781, Oct. 1967.

Writing Committee (Children's Cancer Group A): Preliminary evaluation of daunomycin in children with acute leukemia. *Path. Biol*. 15: 939, Oct. 1967.

Sharp, H.L., Carey, J.B., Jr., White, J.G., and Krivit, W.: Cholestyramine therapy in patients with a paucity of intrahepatic bile ducts. *J. Pediat*. 71: 723-736, Nov. 1967.

Pierce, M.I., Brubaker, C., and Wolff. J.A.: Thiopurines in the treatment of acute childhood leukemia. *Cancer Chemother. Rep*. 52: 321-328, Feb. 1968.

Leikin, S., Brubaker, C.A., Hartmann, J.R., Murphy, M., Wolff, J.A., and Perrin, E.B.: Varying prednisone dosage in remission induction in previously untreated childhood leukemia. *Cancer* 21: 346-352, Mar. 1968.

Krivit, W., Brubaker, C.A., Thatcher, G., Pierce, M., Perrin, E., Hartmann, J.: Cyclic vs. sequential use of chemotherapeutic agents in acute leukemia in childhood. *Cancer* 21: 352-357, Mar. 1968.

Howard, J.P., Albo, V., and Newton, W.A., Jr.: Cytosine arabinoside (NSC-63878) results of a cooperative study in acute childhood leukemia. Cancer 21: 341-345, Mar. 1968.

Krivit, W., Brubaker, C., Thatcher, G.L., Pierce, M., Perrin, E., and Hartmann, J.R.: Study of maintenance therapy in acute leukemia of childhood. Cancer 21: 352-356, Mar. 1968.

Evans, A.: Vincristine (NSC-67574) in the treatment of children with acute leukemia. Cancer Chemother. Rep. (in press).

Sitarz, A., Brubaker, C., Leikin, S., Murphy, M., and Wolff, J.: Induction of remission in childhood leukemia with vincristine and combined 6-mercaptopurine and methotrexate in sequence after failure on prednisone. Cancer (in press).

EASTERN COOPERATIVE ONCOLOGY GROUP

In September 1955 the Clinical Studies Panel established a subcommittee to study the efficacy of several cancer chemotherapeutic agents in human malignancies. The Eastern Cooperative Oncology Group is a direct descendant of this subcommittee, and was originally under the chairmanship of Dr. C. Gordon Zubrod. Dr. Bruce I. Shnider, Georgetown University School of Medicine, has been the chairman of this Group since 1961.

The Eastern Solid Tumor Group was the first Group to be organized to evaluate the use of new chemotherapeutic agents in the treatment of malignant disease by utilizing the cooperative efforts of several large medical facilities. The Group is currently comprised of 81 investigators representing the following 12 member institutions:

- Albany Medical College, N.Y.
  - Veterans Administration Hospital, Albany, N.Y.
  - Ellis Hospital, Schenectady, N.Y.
- Boston USPHS Hospital, Mass.
- Georgetown University Medical School, Washington, D.C.
  - D.C. General Hospital, Washington
  - Veterans Administration Hospital, Washington, D.C.
- Harvard Medical School, Boston, Mass.
  - Children's Cancer Research Foundation, Boston, Mass.
  - Pondville Hospital, Walpole, Mass.
  - Boston Hospital for Women, Mass.
  - University Hospital, Ibaden, Nigeria
- Jackson Memorial Hospital, Miami, Fla.
- Johns Hopkins Hospital, Baltimore, Md.
- Maimonides Hospital, Brooklyn, N.Y.
  - Coney Island Hospital, Brooklyn
- Mary Fletcher Hospital, Burlington Vt.
- Medical College of Virginia, Richmond
- Presbyterian-St. Luke's Hospital, Chicago, Ill.
  - West Side Veterans Hospital, Chicago, Ill.
- Roswell Park Memorial Institute, Buffalo, N.Y.
- Tufts University Oncology Unit, Boston, Mass.
  - Lemuel Shattuck Hospital, Boston
  - Boston City Hospital, Mass.
  - New England Medical Center, Boston

A central operations unit at the chairman's office in Silver Spring, Maryland, provides administrative supervision of the clinical studies and data retrieval and analysis. The latter function had been supplied in the past by the Biometry Branch of the National Cancer Institute.

In addition to conducting its own studies, the Eastern Cooperative Oncology Group collaborates with the Leukemia B Group on studies of mutual interest in patients with lymphomas and multiple myeloma. In response to the emphasis on disease orientation, the Eastern Group is exploring the possibility of collaborating with other cooperative chemotherapy groups in the study of such diseases as hypernephroma and malignant melanoma.

Current investigations include:

Phase I evaluations of Ethidium chloride, Ethoglucid; Mithramycin; combination Procarbazine and radiation therapy; vitamin A; and combination Mitomycin C, 1-Phenylalanine mustard, and Vincristine.

Phase II studies with oral 5-Fluorouracil, Cytosine arabinoside, intermittent parenteral Methotrexate, Mitomycin C, and Natulan.

Phase III evaluations of BCNU in multiple myeloma; combination Cytoxan, Prednisone, and Vinblastine in Hodgkin's disease; combination Cytoxan, Vincristine, and Prednisone on lymphosarcoma and reticulum cell sarcoma; combined radiation and Chlorambucil treatment of ovarian carcinomatosis; Medroxyprogesterone and repository androgen in renal cell cancer; and a dose-response study of Methotrexate in bronchogenic cancer.

Recent Publications:

Kyle, R.A., Carbone, P.P., Lynch, J.J., Owens, A.H., Jr., Costa, G., Silver, R.T., Cuttner, J., Harley, J.B., Leone, L.A., Shnider, B.I., Holland, J.F.: Evaluation of tryptophan mustard (NSC-62403) in patients with plasmacytic myeloma. Cancer Res. 27: 510-515, Mar. 1967.

Hutter, A.M., Jr., Lynch, J.J., and Shnider, B.I.: Malignant testicular tumors in brothers. JAMA 199: 1009-1010, Mar. 1967.

Eastern Cooperative Group in Solid Tumor Chemotherapy.: Comparison of antimetabolites in the treatment of breast and colon cancer. JAMA 200: 770-778, May 1967.

Salmon, S.E., Shadduck, R.K., and Schilling, A.: Intermittent high dose prednisone therapy in multiple myeloma. Cancer Chemother. Rep. 51: 179-187, June 1967.

Cavins, J.A., Hall, T.C., Olson, K.B., Khung, C.L., Horton, J., Colsky, J., and Shadduck, R.K.: Initial toxicity study of sangivamycin (NSC-65346). Cancer Chemother. Rep. 51: 197-200, Aug. 1967.

Hall, T.C., Dederick, M.M., Chalmers. T.C., Krant, M.J., Shnider, B.I., Lynch, J.J., Holland, J.F., Ross, C., Koons, C.R., Owens, A.H., Jr., Frei, E., III, Brindley, C., Miller, S.P., Brenner, S., Hosley, H.F., Olson, K.B.: A clinical pharmacologic study of chemotherapy and x-ray therapy in lung cancer. Am. J. Med. 43: 186-193, Aug. 1967.

Regelson, W., Holland, J.F., Gold, G.L., Lynch, J., Olson, K.B., Horton, J., Hall, T.C., Krant, M., Colsky, J., Miller, S.P., and Owens, A.: 6-Mercaptopurine (NSC-755) given intravenously at weekly intervals to patients with advanced cancer. Cancer Chemother. Rep. 51: 277-282, Sept. 1967.

Foley, H.T., Shnider, B.I., Gold, G.L., Matias, P., Colsky, J., and Miller, S.P.: Phase I studies of porfiromycin (NSC-56410). Cancer Chemother. Rep. 51: 283-293, Sept. 1967.

Salmon, S.E., Samal, B.A., Hayes, D.M., Hosley, H., Miller, S.P., and Schilling, A.: Role of gamma globulin for immunoprophylaxis in multiple myeloma. New Eng. J. Med. 277: 1336-1340, Dec. 1967.

Yount, W., Hall, T.C., Schilling, A., and Shadduck, R.K.: DL-Ortho-phenylalanine mustard (NSC-57199) in the treatment of multiple myeloma. Cancer Chemother. Rep. 51: 517-523, Dec. 1967.

Shnider, B.I.: Future developments in solid tumor chemotherapy. Cancer Chemotherapy. New York, New York, Grune and Stratton, 1967.

Burke, P.J., Lenhard, R.E., Owens, A.H.: Therapy of acute leukemia in adults with cytosine arabinoside (NSC-63878), vincristine (NSC-67574) and prednisone (NSC-10023). Cancer Chemother. Rep. (in press).

Khung, C.L., Hall, T.C., Kelley, R.M., Grogan, R.H., and Griffiths, C.T.: Preliminary clinical trial of DL-metasarcolysin (NSC-27381) for advanced carcinoma of the breast. Cancer Chemother. Rep. (in press).

SOUTHEASTERN CANCER CHEMOTHERAPY COOPERATIVE STUDY GROUP

The Southeastern Cancer Chemotherapy Cooperative Study Group began operations in January 1957 with a group of clinical hematologists representing nine institutions. Dr. R. Wayne Rundles served as chairman of the Group until 1966 when Dr. Charles M. Huguley, Jr. assumed that responsibility. This Group, although interested in neoplastic disease in general, has directed the majority of its activities towards the study of the hematologic malignancies and the malignant lymphomas.

Present Group membership includes investigators in the following institutions:

- Birmingham Veterans Administration Hospital, Alabama
- Charleston Veterans Administration Hospital, S.C.
- Duke University Medical Center, Durham, N.C.
- Durham Veterans Administration Hospital, N.C.
- Emory University, Atlanta, Ga.
- Medical College of Georgia, Augusta
- Presbyterian-University of Pennsylvania Medical Center, Philadelphia
- Temple University School of Medicine, Philadelphia, Pa.
- University of Alabama Medical Center, Birmingham
- University of Kansas Medical Center, Kansas City
- University of Miami, Jackson Memorial Hospital, Fla.
- Washington University School of Medicine, St. Louis, Mo.

The headquarters operations office under Dr. Huguley's direction is located at Emory University. Dr. J. Wanzer Drane directs the statistical center which is also located at Emory.

The following protocols are currently in operation:

- Phase I study oral BCNU in advanced solid tumor.
- Phase I-II study 1-acetyl-2-picolinoyl hydrazine (NSC-68626) for incurable advanced cancer.
- Phase I-II 24 hour infusion of Cytosine arabinoside (NSC-63878).
- Phase II study of BCNU in advanced hematological malignancies.
- Combination therapy in acute leukemia in adults.
- Intravenous high dose Cyclophosphamide in plasma cell myeloma.
- BCNU and CA in advanced solid tumors and acute leukemia.
- Presnisone and Fluoxymesterone as adjuncts to Chlorambucil in chronic lymphocytic leukemia.
- Combination therapy of Hodgkin's disease.

Recent Publications:

Group Studies

Vogler, W.R., Huguley, C.M., Jr., and Rundles, R.W. (Writing Committee for the Southeastern Group.): Comparison of methotrexate with 6-mercaptopurine-prednisone in treatment of acute leukemia in adults. Cancer 20: 1221, Aug. 1967.

Ancillary Studies by Group Members

Durant, J., and Shimkin, M.: Management of chronic leukemia. In Conn, H.F. (Ed.): Current Therapy. Philadelphia, Pa., W.B. Saunders Co., 1967, pp. 44.

Kremer, W.B., and Laszlo, J.: Comparison of biochemical effects of isoprophylidine azastreptonigrin (NSC-72709) with streptonigrin (NSC-45383). Cancer Chemother. Rep. 51: 19, Feb. 1967.

Miller, D.S., Laszlo, J., McCarty, K.S., Guild, W.R., and Hochstein, P.: Mechanism of action of streptonigrin in leukemic cells. Cancer Res. 27: 632-638, April 1967.

Tassoni, E.M., Durant, J., Becker, S., and Kravitz, B.: Cytogenic studies in multiple myeloma: A study of fourteen cases. Cancer Res. 27: 806-809, April 1967.

Laszlo, J., Buckley, L.B., and Amos, D.B.: Infusion of isoimmune plasma in chronic lymphocytic leukemia. Blood 31: 104-110, Jan. 1968.

Vogler, W.R.: 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and cytosine arabinoside (CA) combination in acute leukemia. Proc. Am. Assoc. Ca. Res. 9: 74, Mar. 1968.

Lessner, H.E.: BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea. Effects on advanced Hodgkin's disease and other neoplasia. Cancer (in press).

SOUTHWEST CANCER CHEMOTHERAPY STUDY GROUP

The Southwest Cancer Chemotherapy Study Group has been in operation since 1956 and is currently under the chairmanship of Dr. H. Grant Taylor, M. D. Anderson Hospital and Tumor Institute.

The Group is composed of 90 investigators in the following 19 member institutions:

Baylor University College of Medicine, Houston, Tex.
Cleveland Clinc, Ohio
Emory University School of Medicine, Atlanta, Ga.
Henry Ford Hospital, Detroit, Mich.
Louisiana State University School of Medicine, New Orleans
Northwestern University School of Medicine, Chicago, Ill.
Ohio State University School of Medicine, Columbus
St. Jude Children's Research Hospital, Memphis, Tenn.
Scott and White Clinic, Temple, Tex.
Tulane University School of Medicine, New Orleans, La.
University of Arkansas Medical Center, Little Rock
University of Oklahoma Medical Center, Oklahoma City
University of Texas, M. D. Anderson Hospital, Houston
University of Texas Medical Branch, Galveston
University of Texas, Southwestern Medical School, Dallas
University of Texas, South Texas Medical School, San Antonio
University of Virginia School of Medicine, Charlottesville
Washington University School of Medicine, St. Louis, Mo.
Wilford Hall Air Force Hospital Lackland Air Force Base San Antonio, Tex.

The Southwest Group is divided into an Adult Division under Dr. Robert Talley and a Pediatric Division under Dr. W. W. Sutow. Study responsibilities are further divided among disease committees which supervise group programs for specific types of cancer. A New Agents Committee supervises all Phase I and Phase II studies performed by both divisions. The Group is structured to study all types of cancer, solid tumor and hematologic, in children and adults, in all phases (I, II, III) of anticancer drug investigation.

Currently active studies include:

Phase I evaluation of Hydrazine picolinoyl, Imidazole carboxamide, and Cytosine arabinoside;

Phase II studies involving Hydroxyurea, Melphalan, Mithramycin, 6-Methylmercaptopurine riboside, Porfiromycin, Cytoxan, and various investigational drugs in sarcoma and the hematologic malignancies;

Phase III evaluation of various drugs for induction and maintenance therapy in acute leukemia; a study of CNS leukemia utilizing BCNU and Methotrexate; combination Cytoxan, Vincristine, and 5-Fluorouracil in various solid tumors; a study of childhood malignant lymphomas comparing x-ray, Nitrogen mustard, Cytoxan and Vinblastine singly and in various combinations; a controlled study of Melphalan, Prednisone, and Procarbazine in multiple myeloma; and a remission induction and maintenance study in Hodgkin's disease treated with the combination of Nitrogen mustard, Vincristine, Procarbazine, and Prednisione.

The Group is also actively engaged in the development and evaluation of platelet transfusion techniques and protective environment ("Life Island") procedures.

Statistical services are provided by the Biometrics Section of the Department of Statistics, University of Texas, under the direction of Dr. Edmund Gehan.

Recent Publications:

Haddy, T.B., Nora, A.H., Sutow, W.W., and Vietti, T.J.: Cyclophosphamide treatment for metastatic soft tissue sarcoma. Am. J. Dis. Child. 114: 301-308, Sept. 1967.

Milner, A.N., and Sullivan, M.P.: Urinary excretion of cyclophosphamide (NSC-26271) and its metabolites: Evidence for the existence of an "active" cyclic metabolite. Cancer Chemother. Rep. 51: 343-345, Oct. 1967.

Lane, M.: Animal investigations with cyclophosphamide (NSC-26271): A brief survey. Cancer Chemother. Rep. 51: 359-362, Oct. 1967.

Fernbach, D.J.: Chemotherapy for acute leukemia in children: Comparison of cyclophosphamide (NSC-26271) and 6-mercaptopurine (NSC-755). Cancer Chemother. Rep. 51: 381-387, Oct. 1967.

Holcomb, T.M: Cyclophosphamide (NSC-26271) in the treatment of acute leukemia in children. Cancer Chemother. Rep. 51: 389-392, Oct. 1967.

Sullivan, M.P.: Cyclophosphamide (NSC-26271) therapy for children with generalized lymphoma and Hodgkin's disease. Cancer Chemother. Rep. 51: 393-396, Oct. 1967.

Thurman, W.G., and Donaldson, M.H.: Cyclophosphamide (NSC-26271) therapy for children with neuroblastoma. Cancer Chemother. Rep. 51: 399-401, Oct. 1967.

Haggard, M.E.: Cyclophosphamide (NSC-26271) in the treatment of children with malignant neoplasms. Cancer Chemother. Rep. 51: 403-405, Oct. 1967.

Sutow, W.W.: Cyclophosphamide (NSC-26271) in Wilms' tumor and rhabdomyosarcoma. Cancer Chemother. Rep. 51: 407-409, Oct. 1967.

Luce, J.K., Frenkel, E.P., Vietti, T.J., Isassi, A.A., Hernandez, K.W., and Howard, J.P.: Clinical studies of 6-methylmercaptopurine riboside. Cancer Chemother. Rep. 51: 535-546, Dec. 1967.

Fernbach, D.J., Haddy, T.B., Holcomb, T.M., Lusher, J., Sutow, W.W., and Vietti, T.J.: Uracil mustard (NSC-34462) therapy in children with metastatic neuroblastoma. Cancer Chemother. Rep. (in press).

Fernbach, D.J., Haddy, T.B., Holcomb, T.M., Stuckey, W.J., Sullivan, M.P., and Watkins, W.L.: L-sarcolysin (NSC-8806) therapy in children with metastatic neuroblastoma. Cancer Chemother. Rep. (in press).

Haggard, M.E.: Vincristine (NSC-67574) in acute leukemia of childhood. Cancer Chemother. Rep. (in press).

Sullivan, M.P.: Vincristine (NSC-67574) therapy for Wilms' tumor. Cancer Chemother. Rep. (in press).

Sutow, W.W.: Vincristine (NSC-67574) therapy in malignant solid tumors of childhood (except Wilms' tumor). Cancer Chemother. Rep. (in press).

WESTERN COOPERATIVE CANCER CHEMOTHERAPY GROUP

The Western Cooperative Cancer Chemotherapy Group was organized in 1955 with members representing both University and Veterans Administration Hospitals. In 1960, the VA contingent split off to become a separate entity under the VA Cancer Therapy Program. Dr. Jesse L. Steinfeld of the University of Southern California served as chairman of the Group from 1963 until the end of 1967 when he joined the NCI staff. Dr. Joseph R. Bateman of USC has served as acting chairman since January 1968. The Group is made up of investigators representing the following institutions:

Cancer Research Institute, University of California, San Francisco
City of Hope Medical Center, Duarte, Calif.
Ellis Fischel State Cancer Hospital, Columbia, Mo.
Los Angeles County General Hospital, Calif.
Memorial Hospital, Phoenix, Ariz.
Redwood Community Clinic, Palo Alto, Calif.
Rancho Los Amigos Hospital, Downey, Calif.
Southern California Permanente Group, Los Angeles
John Wesley County Hospital, Los Angeles, Calif.
University of California at Los Angeles Medical Center
University of Oregon, Portland
University of Southern California, Los Angeles, Calif.
University of Utah, Salt Lake City
University of Washington, Seattle

The operations office of the Group is under Dr. Bateman's direction and is located at the University of Southern California at Los Angeles, Statistical support in the past has been provided by the UCLA Computer Center for Health Services under the direction of Dr. Wilfrid Dixon, a member of the Group's executive committee. However, this arrangement will be terminated as of June 30, 1968, and Dr. Bateman will establish a unit within the Group operations office at USC to provide the necessary statistical support. Members of the USC School of Medicine Computer Facility will provide direction and consultation for this unit.

This Group has a broad interest in neoplastic disease and carries out studies of both solid tumors and the hematologic malignancies. Protocols for Phase I, II, and III studies are currently in progress.

During the past year the Group performed Phase I-II studies on solid tumors of Phosphorodiamidic acid (NSC-69945), Procarbazine (NSC-77213), Cytosine arabinoside (NSC-63878), Dibromomannitol (NSC-94100), and Daunomycin (NSC-82151). Phase I-II protocols in acute leukemia were active, employing Daunomycin and Cytosine arabinoside. A Phase II-III study comparing 6-MP, MTX, and combination therapy in acute leukemia was also in progress.

Recent Publications:

Jacobs, E.M., Bateman, J.R., Luce, J.K., and Wood, D.A.: Treatment of cancer with weekly IV 5-fluorouracil. Proc. Am. Assoc. Cancer Res. 8: 34, Mar. 1967,

VETERANS ADMINISTRATION COOPERATIVE CANCER THERAPY GROUPS

The Veterans Administration cooperative cancer therapy groups were organized during the period 1956-1960 as an integral part of the total NCI collaborative clinical chemotherapy research program. Five groups with a total of 150 investigators in 56 VA hospitals now comprise this program. The investigations of these five groups are jointly supported by the NCI and the Veterans Administration. NCI support is accomplished through an annually renewable research agreement under which funds are transferred to the VA Central Office and subsequently disbursed to the investigators.

Membership in the five groups consists of investigators in the following VA hospitals:

Albuquerque, New Mexico
Ann Arbor, Michigan
Atlanta, Georgia
Augusta, Georgia
Bay Pines, Florida
Boston, Massachusetts
Bronx, New York
Brooklyn, New York
Buffalo, New York
Cheyenne, Wyoming
Chicago, Illinois (Res.)
Cincinnati, Ohio
Clarksburg, West Virginia
Cleveland, Ohio
Coral Gables, Florida
Dallas, Texas
Dayton, Ohio
Denver, Colorado
Des Moines, Iowa
East Orange, New Jersey
Fort Howard, Maryland
Fresno, California
Hines, Illinois
Houston, Texas
Iowa City, Iowa
Jackson, Mississippi
Kansas City, Missouri
Lake City, Florida
Livermore, California
Long Beach, California
Los Angeles, California
Louisville, Kentucky
Martinez, California
Memphis, Tennessee
Minneapolis, Minnesota
Mountain Home, Tennessee
New York, New York
Oklahoma City, Oklahoma
Oteen, North Carolina
Palo Alto, California
Philadelphia, Pennsylvania
Phoenix, Arizona
Pittsburgh, Pennsylvania
Portland, Oregon
Providence, Rhode Island
Reno, Nevada
Richmond, Virginia
St. Louis, Missouri
San Francisco, California
San Juan, Puerto Rico
Spokane, Washington
Syracuse, New York
Tucson, Arizona
Washington, D. C.
West Haven, Connecticut
Wood, Wisconsin

With new legislation permitting the VA hospitals to participate directly in the NIH research grant program and as a result of major reorientation of the NCI-Chemotherapy drug development program, it is expected that only two VA groups will continue to be supported through FY-69 under the research agreement mechanism, the VA Lung Cancer Group and the VA Surgical Adjuvant Group. The remaining three VA groups plan to apply for support under research grants.

A brief description and recent bibliography of each group follows:

VA Lung Cancer Group: The VALG was organized in 1958 and is nationwide in distribution. The present group chairman is Dr. Julius Wolf. This Group has studied various forms of chemotherapy and radiotherapy in large numbers of patients with lung cancer. Through large numbers of patients studied and excellent patient follow-up, this Group has produced very complete and reliable results.

The Group's Pathology Panel reviews the tissues on every patient studied by this Group and has made major contributions to the pathology and diagnosis of lung cancer.

Recent Publications:

Wolf, J.: Treatment of the patient with inoperable bronchogenic cancer. Med. Clin. N. Amer. Mar. 1967.

Wolf, J.: Management of the patient with inoperable bronchogenic cancer. Med. Clin. N. Amer. 51: 563, Mar. 1967.

Kaung, D.T., Walsh, W.S., Sbar, S., and Patno, M.E.: Hydroxyurea (NSC-32065) in the therapy of nonresectable cancer of the lung. Cancer Chemother. Rep. (in press).

VA Surgical Adjuvant Group: The VASAG was organized in 1957 and is nationwide in distribution. The present group chairman is Dr. George Higgins. This group studies chemotherapy in relation to various surgical procedures, including the use of anticancer drugs at the time of surgical resection, preoperative chemotherapy and radiation therapy, and chemotherapy administered by perfusion and infusion techniques. This Group is current following in excess of 3000 patients for survival data. The association of a body of radiotherapists with this Cooperative Group has facilitated the development of well-designed protocols incorporating both radiotherapy and chemotherapy in the treatment of patients with lung and colorectal carcinoma. An infusion subgroup has studied in excess of 400 patients and has contributed definitive data to our knowledge of this procedure.

Recent Publications:

Eastridge, C.E., Hughes, F.A., Jr., and Greenberg, B.E.: Primary carcinoma of the lung: Treatment of 1284 cases. Amer. Surg. 33: 700, 1967.

Eastridge, C.E., Hughes, F.A., Jr., and Greenberg, B.E. Evaluation period for lung cancer. JAMA 200: 37, 1967.

Higgins, G.A., and Beebe, G.W. Bronchogenic carcinoma, factors in survival. Arch. Surg. 94: 539, 1967.

Fain, W.R., Conn, J.H., and Chavez, C.M.: Continuous arterial infusion chemotherapy. Amer. Surg. 33: Aug. 1967.

Higgins, G. A.: Preoperative irradiation for lung cancer. Cancer Therapy by Integrated Radiation and Operation. Springfield, Ill., Charles C. Thomas, 1968, pp. 83-86.

Higgins, G. A.: Preoperative irradiation for colorectal carcinoma. Cancer Therapy by Integrated Radiation and Operation. Springfield, Ill., Charles C. Thomas, 1968, pp. 107-110.

Higgins, G.A., Dwight, R.W., Humphrey, E., and Walsh, W.S. Preoperative irradiation therapy as an adjuvant to surgery for carcinoma of the colon and rectum. Amer. J. Surg. 115: 241, 1968.

Higgins, G.A., and White, G.E.: Cancer chemotherapy and surgery. Surg. Clin. N. Amer. (in press).

VA Urology Group: The VAUG was organized in 1959 and is nationwide in distribution. The present group chairman is Dr. George Mellinger. This Group has emphasized the evaluation of hormonal agents in the therapy of prostatic carcinoma and also is conducting studies of radiation therapy in bladder carcinoma and nonhormonal anticancer agents in advanced cancer of the prostate. Statistical support is provided by the Biometry Branch, NCI.

Recent Publications:

Mellinger, G.T., Gleason, D., and Bailar, J.: The histology and prognosis of prostatic cancer. J. Urol. 97: 331-339, Feb. 1967.

Veterans Administration Cooperative Urology Research Group: Treatment and survival of patients with cancer of the prostate. Surg. Gynec. Obstet. 124: 1011-1017, May 1967.

Doe, R.P., Mellinger, G.T., Swaim, W.R., and Seal, U.S.: Estrogen dosage effects on serum proteins: A longitudinal study. J. Clin. Endocr. 27: 1081-1086, Aug. 1967.

Arduino, L.J., and Mellinger, G.T.: Clinical trial of busulfan (NSC-750) in advanced carcinoma of prostate. Cancer Chemother. Rep. 51: 295-303, Sept. 1967.

Veterans Administration Cooperative Urology Research Group: Carcinoma of the prostate: Treatment comparisons. J. Urol. 98: Oct. 1967.

Jordan, W.P., Jr., Walker, D., Miller, G.H., and Drylie, D.M.: Cryotherapy of benign and neoplastic tumors of the prostate. Surg. Gynec. Obstet. 125: 1265-1268, Dec. 1967.

VA Cancer Chemotherapy Study Group: The VACCSG was organized in 1956 and is distributed over the Eastern United States. The present Group chairman is Dr. Henry P. Close. The Group studies all types of anticancer therapies in Phase I, II, and III studies involving both solid tumors and the hematologic malignancies. Drugs currently under study include: TMCA, NSC-17256E, Streptonigrin, Tubercidin, Dibromomannitol, Bonomycin, and Cytosine arabinoside. As a consequence of overlapping areas of interest and patient responsibility, this Group has established close working relationships with the VA Lung Cancer Group.

Recent Publications:

Edwards, G.A., Zawadzki, Z.A.: Extraosseous lesions in plasma cell myeloma. A report of six cases. Amer. J. Med. 43: 194-205, Aug. 1967.

Zawadzki, Z.A., and Edwards, G.A.: M-components in immunoproliferative disorders. Electrophoretic and immunologic analysis of 200 cases. Amer. J. Clin. Path. 48: 418-430, Oct. 1967

Dietrich, F.S.: Clinical trial with dihydroxybusulfan. Cancer Chemother. Rep. (in press).

Pacific VA Cancer Chemotherapy Group: The PVACCG was organized in 1960 as an offshoot of the Western Cooperative Chemotherapy Group. The present Group chairman is Dr. Jack Brook. Although this Group has conducted studies of all types of anticancer therapies in all types of tumors, recent reorganization has increased the emphasis on studies of the hematologic malignancies. Drugs currently under study include: TMCA, NSC-17256E, Cytosine arabinoside, Purin-6-yl-trimethyl ammonium chloride, and Daunomycin.

Recent Publications:

Brook, J.B.: Leukocyte alkaline phosphatase levels in multiple myeloma. Clin. Res. 15: 1967 (abstract).

Brook, J.B., and Lindle, B.H.: Relation of corticosteroids to infection in acute leukemia. Excerpta Medica, International Congress Series 137: 1967.

Phillips, R.W.: Phase I study of purin-6-yl-trimethyl ammonium chloride (NSC-51095). Cancer Chemother. Rep. (in press).

ANNUAL REPORT

PROGRAM ANALYSIS BRANCH

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

The Program Analysis Branch has continued to carry out its function of organizing and disseminating technical information and data on cancer chemotherapy. During the past year, Branch personnel extended their work in data retrieval and systems management in order to provide in suitable form the information needed for program monitoring and to aid in decision making.

Systems Management

1. In addition to their regular duties, all Sections worked closely to provide composite information needed for program monitoring as specified in the linear array. A comprehensive plan is being developed for obtaining the data required for decision making.

2. A new control form was designed and provided to acquire the information required in Preclinical Drug Development.

3. An analysis was made of all shipments of drugs to investigators during the past two years, and cost figures were obtained.

4. The Inventory system was redesigned for improved systems management. Specifications were developed for dealing with investigators requesting drugs sponsored by NCI for investigational use and to meet FDA requirements. It is planned to check each shipment request for protocol compliance. This entails the collection and updating of an extensive data base comprising information on approved protocols, approved investigators, dosage form, etc. Recent developments in computer hardware makes it appear more feasible that we can have a system that will provide easy access to the data and perhaps access to the most important information on a real-time basis.

Program Highlights--Data Retrieval

1. Branch personnel designed and implemented a Clinical Data Retrieval System which provides for the routine accession of limited demographic data on patients admitted to the Clinical Center on NCI services. Information from NCI studies at the VA hospitals and Baltimore are also accommodated as resources permit. Other data banks are being added so that this retrieval system will eventually include a considerable amount of information on all NCI patients admitted to the Clinical Center since its opening.

2. The Laboratory and Clinical Trials Section was reorganized and its function redefined to concentrate on clinical data retrieval. The staff was augmented and became intimately involved in the data management functions associated with implementing this system. Flow sheets and additional data from the clinical records of patients on the Solid Tumor and Leukemia Services have been analyzed, and new methods of retrieval and display have been explored.

3. The application of mathematical techniques, including modeling and sampling, was investigated in a limited way to optimize the information derived from the Chemotherapy Program. This review has led to the decision to implement a trial of processing individual rodent data from the primary antitumor screen. As conversion of the CCNSC Data Processing System neared completion, considerable thought was also given to improved methods of data compression and presentation, including graphical display.

   In cooperation with the Drug Evaluation Branch of CCNSC, steps have been taken to process individual test data in the primary tumor screen as well as for schedule-dependency testing. This will permit future manipulation and analysis in any desired form to reflect changing needs of the program. The data will be transmitted from the test site to NCI via teletypewriters and will be automatically processed during the coming year. The Data Processing Section also centralized an extensive library of all the computer programs prepared both on contract and by staff.

4. The Scientific Records Section assumed responsibility for daily processing of all admission/discharge data on NCI patients.

5. As described in last year's report, the small group in the Office of the Chief is responsible for problem definition, assessment of required resources, and recommendations for carrying out new projects. Considerable flexibility is thus attained, and a central point established for determining the interrelationships of problems and eliminating any possible duplication of effort. Some of the new projects originated in the Office of the Chief in the past year were:

   a. Establishment of a file on patients treated with preserved platelets on the Leukemia Service in the Clinical Center.

   b. Design of the Demographic File for Chemotherapy patients.

   c. Design of an automatic system, including mark-sensing forms, file design, and program design for analysis of the data collected for the Cryopreserved Platelet Study of the Leukemia Task Force.

   d. Design of general-purpose programs for graphical and reportorial display of the data banks established. These have been applied to clinical, toxicology, and primary screening data.

   e. Abstracting Chemotherapy protocols and establishing a file with search criteria built in for ready retrieval.

f. Providing the Drug Evaluation Branch, CCNSC, with a method of graphical display for route-regimen studies performed in the primary screen.

g. Establishment of an inventory file, with search and update capability, of patients and normal donors who have samples of blood plasma and/or serum stored by the Leukemia Service at the Clinical Center.

h. Working in close cooperation with the Clinical Center, particularly with Clinical Pathology Branch personnel (Dr. George Williams and Dr. Ernest Cotlove), to write programs for the selective extraction of clinical laboratory data on NCI patients. These programs permit the preparation of reports over any desired interval by patient name, time period, and type of test. These reports are now available as summary items in the patient clinical record and for other uses. The data can be selected for output in report form, in computer form, or for graphic display.

i. Mark-sensing forms and programs were developed for a comparative study of toxicology in man and other species.

As each of these projects was fully specified and became developmental, responsibility for it was assigned, where staff was available, to other sections. Thus, the Assistant Chief is free to assume the responsibility of investigating new areas and applications and to specify the training needed on a day-to-day basis to carry out new projects.

Program Highlights--General

1. Consultation in data processing, information retrieval, and systems analysis techniques was provided by the Branch and its consultants to all areas of the program. The assistance furnished ranged from providing equipment, such as remote terminals, and designing and furnishing forms for data acquisition, to analysis of data; informal seminars were arranged and an outline of a course has been developed to give Chemotherapy personnel a survey of the Branch's medical ADP applications. The course is scheduled for presentation to new personnel shortly after July 1, 1968.

2. Because of its familiarity with manuscripts, the Publications Section, besides producing Cancer Chemotherapy Reports, was assigned responsibility for manuscript clearance for the entire Chemotherapy Program and broadened its functions to include editing services where desired in the preparation of clinical brochures and other special reports.

3. The Literature Research Section provided much of the information needed for preparation of clinical brochures and for FDA requirements, in addition to their regular duties.

These functions are carried out under the direction of the following staff:

Program Analysis Branch	Chief, Barbara R. Murray
Assistant Chief	Rhoda Mancher
Data Processing and Computer Systems Development Section	Head, Sidney Richman
Laboratory and Clinical Trials	Acting Head, Betty Ann Sullivan
Literature Research Section	Acting Head, Marjory C. Spencer
Publications Section	Head, Evelyn E. Parker
Scientific Records Section	Acting Head, George F. Hazard, Jr.

The work is augmented by contracts in data processing (LEASCO Systems and Research Corporation and Control Data Corporation); data acquisition (Cornell University Medical College and New York University Medical School); in chemical information (Chemical Abstracts Service and National Bureau of Standards); information services (Medical Literature Incorporated, George Washington University, and Japanese Cancer Institute); and statistical services (Emory University, Health Research Incorporated, and Michigan University).

Plans for the Coming Year

1. The Branch will continue to work with the clinical data retrieval system in order to expand its coverage of patients and to add to the flexibility of processing. For example, working in cooperation with the Clinical Center, the Branch intends to explore the feasibility of processing narrative data such as patient discharge summaries. Programs developed under the contract with New York University Medical School for text processing will be used.

2. The improved version of the inventory system will be implemented to provide for checking of approved investigators, etc.

3. Working in cooperation with the Drug Development Branch, CCNSC, we are exchanging data on published compounds with Walter Reed Chemical Information Retrieval System; in return, a master file of structural formulas for use in the Walter Reed structure and inventory systems is being prepared for Chemotherapy use.

4. As the data on individual animals begins to come in from the screening laboratories via teletypewriter, we will explore the problem of permanent storage of data on magnetic tape, update and retrieval capabilities, and the possibility of producing the CCNSC Screening Data Summary as well as other reports and displays directly from the new format. This work will be done in conjunction with the Drug Evaluation Branch, CCNSC.

5. We would like to explore the possibility of using an electronic plotting device to prepare camera-ready copy for publication as an alternative to printed computer output. Perhaps this could be managed in conjunction with the magnetic tape typewriter and an optical character scanning device. This would give the added flexibility of use of any character or figure and of graphical display inserted in the text.

DATA PROCESSING AND COMPUTER SYSTEMS DEVELOPMENT SECTION

I. Responsibilities

This Section is responsible for management and improvement of operating data processing systems, development of new applications, and design of new information systems for the Chemotherapy Program. Technical assistance is available from this Section to all members of the Chemotherapy staff, and data processing support is also provided to the Endocrine Evaluation Branch, General Laboratory and Clinics.

II. Contract Operations

A. Computer-related data processing functions continue to be implemented, under staff direction, by LEASCO Systems and Research Corporation (formerly Documentation Incorporated) under contract PH43-68-30. The contract provides support for the entire Chemotherapy Program and includes, specifically, reduction and preliminary analysis of data developed in the screening programs of the Drug Evaluation Branch, CCNSC, and the bioassay and endocrine-related tumor programs of the Endocrine Evaluation Branch. Other functions include dissemination of recurring reports, preparation of camera-ready copy for Cancer Research Supplement (ceased publication December 1967), and any required programming.

This data processing system has continued to serve the Chemotherapy Program by the reduction and storage of test data and selective dissemination of information. Screening test results are automatically validated, calculations are made, information is stored for future retrieval, and a variety of reports are distributed to suppliers, testing laboratories, and NCI scientists. The publication subsystems have also provided for automatic selection and retrieval of information from the data bank and the computer generation of camera-ready copy for the publication of test results.

B. Under contract PH43-65-1021, Chemical Abstracts Service is developing and operating an automated NCI chemical registry system. The registry provides information on NCI compounds with regard to chemical structure, names, and bibliographic references. This Section identifies users' information needs and monitors the systems development aspects of the contract.

III. Highlights of the Data Processing Systems

A. General

Evaluation of the Drug Evaluation System basic programs continued during the year, and rewriting of the programs to operate in the IBM System 360 was essentially completed; this effort is described below. Much of the work was carried out in COBOL so that the programs can be essentially machine-independent.

Program documentation was formalized, a program library was established, and a number of programs were validated and tested. An extensive procurement effort was made to secure broad competition for the award of a data processing contract to serve the Chemotherapy Program for future periods.

B. Conversion to the System 360

1. Programming. The bulk of the programming effort for the conversion of the CCNSC system to the System 360 was completed by PRA programmers. In addition to the four major programs (Cell Culture Calculation, Validity, Update, and Print and Punch) required for the processing of experimental data, several additional programs were written to reformat the files, to add test descriptors to the data, to merge split files, and to create subfiles consistent with the converted system.

2. Forms Design. All input and output forms were revised and designed to conform to the converted system.

3. Procedures Manual. An extensive procedures manual for the converted data processing system was prepared, covering activities of the suppliers, screeners, data processing contractor, and PRA. This manual will serve as a basis for training personnel in the converted system and for orienting any new data processing contractor.

4. Microfilm Library of Summaries. With the exception of Screening Data Summaries for the Selected Agents, the library of 250,000 summaries will be reproduced in the revised format on microfilm directly from magnetic tape. The film will be so indexed that the frame for a given summary may be located in seconds by the use of microfilm image control equipment. This procedure of storing the bulk of the Screening Data Summary Library on microfilm will decrease the amount of storage space required as well as eliminating the considerable computer time entailed in reprinting the Library.

C. Monitor the LEASCO Systems and Research Corp. Contract

Highlights of this activity follow:

1. Drug Evaluation Data Processing System

   a. Queries of the Files. A generalized selection program was developed to query magnetic tape files for varying criteria (such as activity, toxicity, or incomplete testing in each test system) or to select compounds by chemical structure classification codes. Examples of queries include:

(1). List the NSC numbers for compounds entering the screen in 1967, which met prescribed activity levels in L1210 testing, or which were not tested in L1210, or for which L1210 testing was not completed.

(2). List compounds, shipped prior to 1967, for which there is no record of shipping nor test data.

(3). For selected compounds of interest, list the test systems in which the compound met specified criteria of activity in survival and solid tumor systems; for selected test systems, list the test results by compound for designated dose regimens and routes.

(4). List non-discreet compounds tested in 3LE (single treatment, day 2) at a dose of 100 mg/kg with toxic results, excluding positive controls.

In addition several major inquiries were made of the file to produce Screening Data Summaries for use in structure-activity correlation studies. One example is an inquiry for approximately 1,600 quinones and tetracyclines.

b. Modification of Preconversion Programs. Several programming changes were made to the 1401/1410 programs to meet operational requirements of CCNSC which could not await the conversion of these programs to the System 360. A typical example is the modification of several programs to accept and display an externally assigned evaluation of dose response testing.

c. Programming Assistance for Conversion Effort. A program for preparing the SAC (Selected Agents) file was written for the 360. Although this file had been established during the last fiscal year, conversion and reprogramming of the CCNSC data processing system necessitated revisions because all existing data files are being merged and converted to the format of the new system.

The value of the Selected Agents File, established in FY 1967, continued to be demonstrated during 1968. Searches of the file which required reference to the total store of information on SAC compounds were made in a more efficient manner (with respect to computer usage) and with more powerful utilization of the logical capabilities of the program. Further, use of this file yielded unified reports rather than separate reports from each subfile (Weekly Processing File and Consolidated File) which would require subsequent consolidation.

d. Programs for Selection, Alphabetization, and Printing of Plant Products. Programs written in PL-1 were developed to prepare listings of plant products for collections in Arizona, California, and Costa Rica. These programs are sufficiently generalized so that, with minor modifications, they will perform similar functions on any card file. The only change in the program required is to designate the location of the field to be selected or alphabetized in the input cards.

e. Publications

(1). Cancer Research Supplement (CRS). Computer-generated, camera-ready copy was prepared for eight issues of CRS for a total of 1,307 pages and reporting test data for 11,716 synthetic or plant products.

(2). Nucleosides. A 300-page report was prepared and reproduced for distribution at a working conference on retrospective analysis of the structure-activity relationships of nucleosides.

(3). Devlin Paper. Existing programs were modified to prepare computer-generated, camera-ready copy for the publication in the CRS of Dr. T. M. Devlin's paper, "Screening Data from Selected In Vitro Enzamatic Screens, I and II." This change demonstrated the flexibility of the CCNSC publication system.

f. Clinical Brochures and Special Reports. Nine clinical brochures, 7 toxicology reports, and minutes of one Clinical Trials Task Force meeting were prepared for publication and reproduced.

g. Report Survey. All recipients of computer-generated reports were canvassed by questionnaire to establish their specific needs for reports. This information is being added to the "report-to" code on the 360 System magnetic tape file and the Print and Punch Program is being modified to produce for each recipient of reports only the printed Screening Data Summary or punched-card Interim Screening Test Report as required. This change eliminates the generation of undesired information and also reduces the computer usage required for the CCNSC system.

h. Declassification of Compounds. Prepared release sheets containing nomenclature information for 10,000 compounds to be mailed to suppliers requesting declassification action.

i. Squibb Data Magnetic Tapes. Improved the methodology for providing a magnetic tape extract of data for Squibb compounds from the CCNSC file. Programs developed for other purposes were sufficiently generalized to extract data for Squibb compounds new to the CCNSC file for updating the previous Squibb file. Tapes were furnished quarterly to Squibb for inclusion in the supplier's data bank.

j. Sloan-Kettering. Developed a punched card file for information extracted from Sloan-Kettering test reports and chemistry cards. Prepared a variety of reports, including one for "active" Sloan-Kettering compounds not tested by CCNSC which was used for procurement and testing action.

k. Graphics. Provided graphics support in the revision of the basic computer output forms of the CCNSC system to conform to the requirements of the data processing system being converted to the System 360.

2. Endocrine Evaluation Branch

a. Programming

(1). Publication programs were generalized to provide the capability of selecting test data and pertinent compound information onto magnetic tape. These programs can be used to prepare camera-ready copy for all assays in the form of cumulative indexes.

(2). Several program modifications were made to meet the Branch's operational requirements, including the capability of printing test results for five test objects rather than three and of processing data related to cures.

b. Publications. Much of the work required for the preparation of three issues of Endocrine Evaluation Data for Assays 02, 06, and 08 was completed. These reports will be published early in FY 1969.

c. Squibb Data Magnetic Tapes. Furnished extracts of data for Squibb compounds on magnetic tape to the supplier for inclusion in his data bank.

d. Systems Study. A systems study was completed to assess the limitations of the current data processing system in meeting the needs of the Branch and to recommend changes in design or modification of parts of the system. The report of the study recommended redesign of the endocrinology system for expanded analysis procedures and to improve the reporting format so that it may be a means of "screening" for new combinations of activity, for detecting structure-function relationships, and for obtaining data useful for advance guidance on physiological and pharmacological dosages.

3. Documentation. The documentation of all programs developed by the contractor and staff was reviewed and consolidated, and a program library was established at PRA. This library is proving invaluable as a reference when changes are needed to programs. In addition, the library (1) provides a rapid method of reviewing systems concepts without having to learn the details of the program; (2) enables a user to determine whether a program suits his need or whether it can be modified easily to provide him with the necessary information; and (3) provides concise operating instructions in a rapidly accessible format.

IV. Monitor the Chemical Abstracts Service (CAS) Contract

Highlights of this activity follow:

A. Editing of Chemistry Cards. Edited 73,900 chemistry cards for consistency in structure, nomenclature, and molecular formula and assigned current CAS nomenclature. A total of 131,000 chemistry cards were edited by the close of 1967, including some 5,000 Sloan-Kettering compounds assigned NSC numbers.

B. Structure File. Encoded and registered the structure in the form of a condensed connection table on a magnetic tape file. From the start of the contract through 1967, a total of 126,000 structures have been processed and 119,241 unique structures registered. This structure file is intended for use with a substructure search system to be completed in 1968.

C. Bibliography Files. The CAS 360 Bibliography File System was modified to provide information required by NCI for each NSC, in addition to the registry number, molecular formula, and nomenclature. During 1967, a total of 57,800 compounds were registered on this file, bringing the file size to 125,600 compounds.

D. Desktop Analysis Tools. The CAS 360 Desktop Analysis Tools System was modified to meet NCI requirements on the preparation of indexes of NCI compounds arranged by nomenclature and molecular formula. These indexes will include the following information for all registered compounds: NSC number; NCI registry number; molecular formula; nomenclature, both CAS-approved names and other names, identified as to name type; the code for the supplier of the compound; discreet code; publication status; and CAS registry number for published compounds. These indexes will permit access to information by name or molecular formula and provide a fast reference to determine whether a compound is in the Chemotherapy Program.

E. Preparation of Chemistry Cards. Approximately 2,300 chemistry cards were prepared by CAS from data sheets provided by suppliers submitting new compounds for testing in the primary screen. The purpose of this work was to explore the feasibility of having this type of work done under contract and to see if any savings could be effected by capturing the information on chemical compounds in machine-processable form as early as possible in the cycle.

F. Present Status of Contract. A four-month extension of the contract was arranged in order to evaluate the CAS effort and its contribution to the Chemotherapy Program. The criteria of evaluation will be the accuracy of the CAS-assigned nomenclature and related information on the NCI Bibliography File and the validity and costs of operation of the programs for the NCI Structure File, Bibliography File, and Desktop Analysis Tools systems. Selected sample records from the Bibliography File will be reviewed against NCI sources of information for verification of accuracy. As soon as all magnetic tape files and related data have been received, these programs, as documented by CAS, will be evaluated with the assistance of DCRT on the NIH computers. These findings will aid in determining the future work required under this contract.

V. Objectives for the Next Fiscal Year. With the completion of the conversion to the System 360 of the CCNSC data processing system, efforts will be directed toward the enrichment and increase in efficiency of the system. Typical projects will include: (1) the analysis of the mortality distribution of experimental animals by nonroutine statistical methods to signal atypical patterns and to plot data as required; (2) the analysis of tumor growth with time to establish the rate of change as compared to controls and to express the effects of treatment in terms of percent cell kill; (3) the evaluation for extension to other major screeners of the experimental transmission of test data from screening laboratories to NCI by teletypewriter for automatic conversion to a machine-processable form; and (4) the revision of the publication programs to handle data on compounds showing activity in the primary screen as opposed to the conventional methods of preparing tables of negative data.

An evaluation will be made of the interface capabilities of the Chemical Abstracts service, including the newly developed System 360, substructure search, with the information requirements of the Chemotherapy Program and the capabilities of alternate chemical information systems, utilizing Wiswesser linear notations.

When staff and time become available, the Section will also assume responsibility for processing those clinical applications which have become sufficiently well defined and are of a recurrent nature. A typical example is the processing of mark-sensing forms such as are used in the collection of data from a study comparing frozen with fresh platelets.

LABORATORY AND CLINICAL TRIALS SECTION

I. Responsibilities

In the past, the principal responsibilities of the Laboratory and Clinical Trials Section had been the monitoring of contracts with Regional Statistical Offices and the operation of the Uniform Reporting System--a method for obtaining data on cooperative group patients in clinical trial.

During the past year, the functions of the Section have been redirected toward clinical data retrieval for patients within the Chemotherapy program.

A. Contract Operations

This Section was responsible for working with the Regional Statistical Offices, each of which provides statistical services for one or more of the cooperative study groups in the Chemotherapy Program. Three Regional Statistical Offices remained under contract during this fiscal year; however, as each of the other contracts terminated, the change was made to grant support.

B. Uniform Reporting System

This system is the mechanism used for collecting data from the cooperative groups on protocol studies of NCI-sponsored drugs. Although the Uniform Reporting System worked well during this period, processing the data for which it had been designed, the inherent disadvantages common to any report comprising summarized data became more obvious. Despite extensive attempts to redesign and revitalize the reports, there was insufficient staff time available for the detailed planning necessary not only at NCI but in the statistical offices of the cooperative groups. Consequently the system was discontinued at the end of 1967, and the staff of the Section was deployed so that all efforts could be directed toward clinical data retrieval for the Chemotherapy program.

During FY 1968, the Uniform Reporting System was established on a current basis, with monthly or bimonthly reports to each cooperating study group on the status of its respective reports, including follow-up of delinquencies, data-screening diagnostics, file maintenance diagnostics, clinical operations and abnormalities summaries, and masterfile printouts. Two special tabulations were produced, by primary tumor site, in the formats of the clinical operations summary and the clinical abnormalities summary.

When the study was terminated, complete data were on file on 22,578 cancer patients, with on-study data only on an additional 7,778 patients. Full documentation of the system is on record, and the most recent updated data are being retained on magnetic tape.

II. Clinical Data Retrieval

A. Special Study Files

At the start of this report period, a small group within the Section began to work with individual complete protocol data from the Solid Tumor Service using an available information retrieval system, INFOL.

Files have been established for two studies on the Solid Tumor Service in order to provide as much computer-assisted analysis of data as possible. All observations, treatment, and laboratory results required, as well as narrative comments by the physicians, are entered into the file. Programs to calculate various forms of toxicity and response status and to determine protocol compliance (ie, to check that laboratory data have been collected at specified intervals) have been tested and are now being studied by the physicians for refinement. Graphs comparing patient reaction and administration of drug are prepared by computer upon request. Procedures for producing reports summarizing patients on a study by dose, schedule, diagnosis, total amount of drug received, and total length of time on study are being tested with the goal of enabling the physician to request a desired report by simply transmitting by teletype directly to the computing center.

For one of these studies patient reaction, physical findings, therapeutic agents, and a few laboratory results are being prepared on mark-sensing forms. The remaining laboratory results are obtained by extraction from the Clinical Center Clinical Pathology magnetic tape files or in the case of non-Clinical Center patients, from the comments section of the mark-sensing forms.

B. Master File of NCI Clinical Center Patients

In October 1967 the Branch was directed to have an operational data retrieval system by 1 January 1968. Preliminary work which had been carried out by an ad hoc group consisting of the Branch Chief, the Assistant Chief, and the few programmers available from the Laboratory and Clinical Trials and Data Processing Sections had by then progressed to the point where the system was fully defined. As the work became routine, responsibility for the master file was placed in the Laboratory and Clinical Trials Section.

Using as a base an existing file of the most recent admissions and discharges of patients in the Clinical Center during 1963-1966, a demographic file of all NCI patients admitted to the Clinical Center was established. In addition to dates of admission, discharge, and death, diagnoses have been added and are being updated where appropriate. The planned therapy (in the form of protocol numbers) and specific NSC drugs administered are being prepared for Leukemia Service patients for addition to the file. Information on a few VA Chemotherapy patients has recently been added to the master file.

Data prepared for the file is for patients currently on study, while retrospective information and information from other data banks is being collected as resources permit. When the original diagnoses and corresponding dates for all patients have been collected, survival reports with breakdowns by diagnosis can be generated. For patients with protocol and NSC numbers, the same reports could be prepared by treatment. This master file is designed to store pointers to files containing additional data; one such file of Clinical Center laboratory data is being updated weekly.

C. Protocol Abstract File

Study group protocols are abstracted for computer processing so that they can be selected by group, protocol number, drug name and NSC number, tumor site or disease, or any combination of these. Independent protocols are being studied to determine in what respect they differ from those from the study groups in items of information available and required for reporting. Abstracts of NCI protocols are also used in the computer programs to check protocol compliance in terms of criteria for patient selection, required observations, and laboratory tests.

D. Data Acquisition Methods

Punching data onto paper tape directly from records and transcribing data onto prepared forms were the first two methods investigated in an effort to determine the best way to collect data from various types of patient record service documents. Abstracts of protocols have been prepared for processing by keypunching and by papertape via remote entry; and the use of optical scanning of typed sheets is being explored on a limited basis. Mark-sensing forms are also being used on an expanding basis. The emphasis on source data automation saves time for all and results in greater accuracy.

III. Plans for the Coming Year

As the Laboratory and Clinical Trials Section takes a more active role in support of clinical studies, the areas described above will be emphasized since they are representative of the types of data to be processed, the associated problems of obtaining the data in a form permitting computerized processing, and the kinds of retrieval that are proving useful. The Section will continue to support the Chemotherapy clinical activities by meeting specific problems and developing new areas in both data acquisition and processing as time and resources permit.

LITERATURE RESEARCH SECTION

The Literature Research Section is an information center serving the cancer chemotherapy investigators. The Section provides Chemotherapy staff with information from the literature and, when applicable, pertinent data generated by the Cancer Chemotherapy Program and its contractors. Information is also supplied to the FDA in support of the Investigational New Drugs (IND) sponsored by the NCI.

As an aid in this work, the Section also maintains a photo library of the world's literature on chemotherapy; these copies are currently secured through the contract with Medical Literature, Inc., as a by-product of the preparation of Cancer Chemotherapy Abstracts (CCA).

Literature Services

During the year the Section concentrated on: 1) providing specialized searches and survey services; 2) attempting to facilitate the use of its photo library by incorporating the literature (through contract) into an automatic microfilm reader-printer system; and 3) improving the quality of the CCA service.

The Section filled approximately 95 requests for searches of the literature during the year ending April 15, 1968. Sources for the searches, which frequently involve a much broader scope than cancer chemotherapy, have included the unpublished data generated by the Cancer Chemotherapy Program as well as the open literature. Information was provided in the form of annotations or abstracts, copies of articles, and data compilations or summaries.

A major part of the requests involved searches of the literature on potential step IVA compounds; these searches provide background needed in decision network review, in writing clinical brochures, and in meeting the FDA requirements for IND filing.

The most comprehensive undertaking of the year was the completion of an annotated bibliography of 545 references on choriocarcinoma and related diseases. The bibliography, which was selective, covered a 25-year period from 1943 to 1967. All significant reports on etiology, epidemiology, incidence, and chemotherapy of the diseases were included; as requested, representative contributions in the areas of natural history, biologic behavior, classification, diagnosis, pathogenesis, prognosis, and metabolic studies were also added. Other extensive searches included compilations on the treatment of Hodgkin's disease, treatment of carcinoma of the bladder, long-term remissions in acute leukemia, and the cardiac toxicity of cancer chemotherapeutic agents.

The long-range project of searching the literature for known constituents of plants, which was initiated in 1966, was continued as time was available; the literature on 209 species of 122 genera was searched for their respective compounds.

Contract Operations

The Section is responsible for monitoring three service-type contracts: the Cancer Chemotherapy Abstracts, the Japanese Cancer Institute literature contract, and the George Washington University microfilming and indexing contract; all three contracts are designed to augment the services of the Literature Research Section.

Cancer Chemotherapy Abstracts, a current awareness serial, has a twofold purpose. It provides the investigator with abstracts of the world's literature in the field of cancer and supplies the Literature Research Section with hard copy of all the papers abstracted or cited. Special effort has been exerted throughout the year in directing the development of terminology controls for the service. Direction in the establishment of a nomenclature file for indexing chemical compounds and in the use of standard codes for indexing human tumors has been provided, as well as guidance in the overall improvement of its quality.

The primary objective of the Japanese Cancer Institute contract is to provide translations and abstracts of Japanese reports in the field of cancer chemotherapy for incorporation into the Section's retrieval system. Particular attention has been directed to the contractor's scanning in an attempt to increase the yield of pertinent literature.

The George Washington University contract has two purposes: 1) rapid scanning and easy retrieval of hard copy of the cancer chemotherapy literature by incorporating into an automatic microfilm reader-printer the documents cited in CCA from 1960 through 1966; and 2) providing a hard-copy cumulative author and agent index to the literature for these years. Much effort has been exerted in planning, organizing, and monitoring this project which will effect considerable savings in space and will be available to users of the chemotherapy library on a self-service basis.

The Section has drawn on the resources of other groups in order to supply the comprehensive and highly integrated responses the requests demand, particularly the Data Processing and Scientific Records Sections of the Program Analysis Branch, and has worked closely with the National Library of Medicine in its development of a Cancer Chemotherapy Bibliography.

PUBLICATIONS SECTION

The principal activity of this Section during the past year has been the publication of clinical and laboratory reports through the medium of Cancer Chemotherapy Reports (CCR), which is now in its 10th year of continuous publication, with a total to date of 66 issues. This activity is part of the information system developed under the Cancer Chemotherapy Program from the original recommendations of the Chemotherapy Committee of the National Advisory Cancer Council. In 1959, CCR supplanted the original newsletter called "Current Research in Cancer Chemotherapy," which was first published in 1954.

The increased acceptance of CCR among scientists, here and abroad, has led to increased requests to CCR for the publication of papers presented at conferences so that the information will have a wide circulation. Special issues to accommodate some of these requests, this past year, have been "The Symposium on Cyclophosphamide in Pediatric Neoplasia," October 1967, Volume 51, No. 6; "Conference on Lymphography," January 1968, Volume 52, No. 1; and the "Vincristine Symposium," which will be published in June 1968, Volume 52, No. 4.

The following table shows the number of manuscripts received and the number accepted for publication in CCR during 1967 in comparison to previous years, and other pertinent information:

Summary of data on CCR--1964-1968

	1964	1965	1966	1967	1968 (Jan 1-Apr 15)
Manuscripts received	148	102	96	125	40
Manuscripts accepted	89	66	44	87	19
Issues published	10	6 (3 vol supp)	9	7	2
Pages published	758	413 1528 supp	707	561	329
Reviewers	76	114	121	143	53

The following new projects have been assigned to the Publications Section:

1. Clearance of Contractor's reports.

   From July 1, 1967, through March 31, 1968, 155 publications from contractors were cleared.

2. Miscellaneous editing assignments such as clinical brochures and toxicology reports.

3. *Part 2 Supplement to Cancer Research* which will be published in the future as a Supplement to *CCR*.

4. Program Information will also be published as a Supplement to *CCR*.

The Publications Section continues in its responsibility of answering requests for current and back issues of *Cancer Chemotherapy Abstracts*, *Cancer Research Supplement*, and *CCR* and for maintaining these mailing lists. Requests to receive these publications regularly are carefully screened according to the criteria established. The present distribution of *CCR* is as follows:

	3950	total foreign and domestic mailing
	200	NIH (local mailing)
	47	to Publications Section
	87	GPO files
	322	Superintendent of Documents (for Depository Libraries)
Total	4606	

SCIENTIFIC RECORDS SECTION

I. Responsibilities

The Scientific Records Section provides staff support to the Chemotherapy Program with particular emphasis on the work of the Drug Development Branch and the Drug Evaluation Branch. The Section maintains the master files on synthetic chemicals and natural products, operates a chemical search system, and is responsible for the filing of computer-generated biological test data. Duties assumed during the last year have been in support of the Cancer Therapy Evaluation Branch (distributing cooperative group reports, minutes, and protocols) and of the clinical data retrieval system (processing routine clinical data). Other duties include preparation of data processing inputs and outputs, maintaining lists of eligible investigators, Investigational New Drugs, and mailing labels for cooperative groups through the data processing contractor.

A. Storage and Retrieval

The National Bureau of Standards is modifying the Microcite, a microfilm device used with the optical coincidence (Peek-a-boo) system for substructure searching. Changes to the equipment will allow direct readout of the NSC numbers of desired compounds on punched cards which can then be used as input to a computer as a first step toward mechanizing structure-activity correlations. The engineering changes will be completed by the end of FY 1968.

Operation of the substructure search system was improved during the past year by the filming of two additional microfilm matrices representing 36,000 compounds for use with the Microcite. Another 18,000 compounds are to be filmed in the near future as a step toward full coverage of the file. This will greatly speed analog searches by allowing direct inspection of the image of compounds turned up by specific search parameters, rather than manually listing NSC numbers and then eliminating inapplicable compounds after reviewing the chemical structures.

Thirty-seven major substructure searches have been made since last April, together with 20 updates of previous searches and 11 supplier's searches. As an example, a search of all quinones in the program was made and copies of the chemistry cards and screening data summaries were sent to the Drug Development Branch for a structure-activity study. A similar search was initiated for all alkaloids, with emphasis on those containing fused nitrogens. Other representative searches were for asparagines, adamantanes, lanosterols, proteins, and various other compound types in which Chemotherapy staff expressed interest.

The Section also maintains two separate Peek-a-boo files for active compounds, one for all active classes and the other for step IV-A compounds. These files were used mainly for the 150 active searches requested by the Drug Evaluation Branch for analogs of promising new step III compounds to determine their structural uniqueness. This information is one of the factors considered by the Drug Evaluation Committee in their decision whether to recommend these compounds for step IV-A.

A study was made of the changing accession rates since the beginning of the program, of alkylating agents, nucleosides, and various other classes of agents. All ring systems new to the program this year were indexed according to structure (based on the Revised Ring Index number), and the compilation was printed. A similar survey made for all active rings gives the structural formula, the total number of compounds containing a particular ring system, the total number of step IV-A compounds for each ring, and NSC numbers.

Other recurring search duties included those made to help in evaluating 100 research grant proposals (300-500 searches) and in finding NSC numbers and acceptable synonyms for compounds mentioned in Cancer Chemotherapy Reports.

A three-letter family code was assigned to all active compounds describing general chemical class, substituent type, and ring size. These codes are being added to the Selected Agents File maintained on magnetic tape for computer processing.

B. Duties of Supporting Staff

About 7,500 compounds were processed last year in the Section. For each compound, several clerical steps were necessary: hand drawing the structure; punching and proofreading a Flexowriter (automatic typewriter) tape of nomenclature and other information; and making the shipping and suppliers lists, to be used for sending out compounds and for computer inputs. A faster and more versatile Flexowriter purchased this year has helped to speed this process and it is hoped that future purchases of this model will cut down on retrieval times even more.

Two large publications during the year necessitated running about 2,000 Flexowriter tapes. These were "Plants Used Against Cancer, A Survey," by Dr. Jonathan Hartwell, DDB, and a survey on nucleosides in the program, by Dr. Goldin et al, CCNSC, NCI. Tapes of nomenclature approved by the Chemical Abstracts Service were routinely run for compounds mentioned in manuscripts submitted to Cancer Chemotherapy Reports. Structural and molecular formula corrections found by Chemical Abstracts Service under contract were made for a series of 36,000 compounds to prepare them for microfilming for the Microcite.

As part of the Section's services to other branches, 8,000 natural product cards were files for the Drug Development Branch and about 120,000 Screening Data Summaries were added to the biological testing library in the Drug Evaluation Branch. On an average, 100 documents a day, mainly chemistry cards and Screening Data Summaries, were duplicated for various requestors. A new service this year was the mailing of data from cooperative groups for the Cancer Therapy Evaluation Branch. About 3,000 of these documents were distributed.

C. Data Processing

In cooperation with the Data Processing Section, the Section's computer-oriented work was flow-charted, and several suggested changes are being studied to streamline operations. Section personnel are being familarized with the changes resulting from modification of the CCNSC data processing system.

In the routine checking of computer error outputs for synthetics and plant products, about 900 worksheets to update master records were processed, and the results checked for keypunch accuracy. Similarly, there were 350 worksheets used to update the off-line card files for such documents as the Selected Agents List, the QNS (Quantity not Sufficient) lists, Confirmed Active Plants, etc. Each of these worksheets includes from 10 to 20 lines of input data.

The computer input for the clinical drug inventory system has been prepared by the Section. The work involves checking the clinical drug shipment requests and preparing the data in the format used for keypunching, as well as proofreading the outputs.

Several Section employees familiar with the Flexowriter were trained in the use of a computer remote (teletypewriter) and have assumed responsibility for processing routine patient entry and discharge data on a daily basis.

II. Future Plans

In the next year, efforts will be continued to streamline the Section's operations to better serve Chemotherapy personnel. The chemical search facilities will be improved by completion of the microfilming of the entire file of chemistry cards for use with the Microcite for substructure searching; other areas of chemical documentation will be explored; and the chemistry card file will be modified to conform to the changes made under contract by the Chemical Abstracts Service.

Additional processing of clinical data will be assumed, and Section personnel will be trained in the use of special-purpose data-acquisition techniques.

SUMMARY REPORT

ASSOCIATE SCIENTIFIC DIRECTOR FOR CLINICAL TRIALS

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

July 1, 1967 -- June 30, 1968

In broad terms, the intramural and contract activities of the Clinical Trials Area during FY 1968 can be classified into four major categories identified in the NCI Chemotherapy Program Linear Array:

(1) Initial clinical trials of anti-tumor agents in man (Stage V, Step 1A)

(2) Supportive care of patients undergoing intensive anti-tumor therapy (Stage V, Step 1B)

(3) Delineation of therapeutic activity of agents in rapidly and slowly growing tumors (Stage V, Step 2)

(4) Tumor eradication (Stage VI, Step 2)

(5) Studies on cell regulatory mechanisms. This work is related to several areas in the Linear Array (Stage 1, Stept 1 and 2; Stage V, Step 2, etc.).

(1) INITIAL CLINICAL TRIALS OF ANTI-TUMOR AGENTS IN MAN (STAGE V, STEP 1A).

Studies are in progress to evaluate several new agents. NSC 82196, Imidazole carboxamide bis-β-chlorethyl triazeno, a compound with high activity against murine leukemia L1210 has been given to a small number of patients with acute lymphocytic leukemia and to 13 patients with a variety of solid tumors. To date no therapeutic effect has been observed but levels of limiting toxicity have not yet been reached. These studies are to continue.

The most exciting of the new agents, at least from a conceptual standpoint, is L-asparaginase which has recently been obtained for clinical use at NCI. Quantities are still limited but 4 patients in the Leukemia Service and 4 in the Solid Tumor Service have been treated. One adult and one child with acute lymphocytic leukemia and one patient with melanoma have shown significant responses. Studies in the coming year will include an evaluation of the drug for remission maintenance capability, schedule dependancy, effect on residual disease, and combination therapy.

New agents to be studied in the coming year include trifluorodeoxythymidine, streptozotocin, and chlorocyclohexyl nitrosourea.

(2) SUPPORTIVE CARE (STAGE V, STEP 1B)

A major element in the improved results in acute lymphocytic leukemia is unquestionably due to the advances in the supportive care of these patients.

Severe thrombocytopenia is treated aggressively with platelet transfusions and the incidence of hemorrhage due to thrombocytopenia has decreased significantly as a consequence. Fresh platelets for patient care at NCI are obtained from the American Red Cross (64-102) and the Central Blood Service (Baltimore). Obviously, the problem of supply would be far easier if stored preserved platelets could be used. A comparative study of fresh and frozen platelets got underway during FY 1968. Frozen platelets are provided under contract primarily by the Children's Cancer Research Foundation (66-1147) and the Children's Hospital of Philadelphia (66-1152) and to a lesser extent, by the American Red Cross. This is a cooperative study with members of the Acute Leukemia Task Force and the first two institutions mentioned above provide the frozen platelets.

Although the techniques of platelet preservation have been known for some years, suitable containers have not been available. However, during the year a satisfactory plastic container was developed and produced in adequate numbers under a contract with Travenol (67-1403).

Infection still remains the major cause of death of patients in the Leukemia Service with gram negative organisms largely responsible. Several studies are in progress to evaluate the efficacy of new antibiotics such as gentamycin. Large numbers of leukocyte transfusion continue to be given and although there is good suggestive evidence that they are of value, the beneficial effect has never been clearly shown. The majority of the leukocyte transfusions are from donors with chronic myelocytic leukemia (CML) but their availability is variable. Fortunately, during FY 1968 a most exciting objective was finally achieved which will solve the supply problem. The NCI-IBM Cell Separator developed under a contract with the International Business Machines Corporation (64-100) became operational. During the last 8 months of the year white blood cells for the supportive care of leukopenic patients were obtained solely with the Cell Separator. Approximately 100 runs using CML donors were performed with the procurement of a minimum of 10" (approximately 100 gms.) leukocytes per hour. Since no untoward incidents occurred with the CML donors, authorization was granted for the use of normal donors (relatives of youngsters with acute leukemia).

In the coming year, the primary efforts in this program will be to increase the efficiency of the Cell Separator, to develop a technique and a centrifuge bowl for the preparation of platelet concentrates, and investigate techniques for platelet and leukocyte preservation.

The program to evaluate the effect of a germ-free environment ("Life Island") in the supportive care of patients undergoing intensive cancer chemotherapy continued during FY 1968. Substantial evidence was obtained that the incidence of infection in leukopenic patients who are at high risk to infection is markedly reduced in the isolator unit.

An important study was initiated comparing the course of untreated patients with acute myelogenous leukemia (AML) within the isolator unit with patients outside the unit undergoing identical treatment. This disease was chosen since a number of studies have now shown that a majority of AML patients who do not achieve remission die of infection early in the course of therapy.

The present isolator unit presents a number of problems both practical and psychological. The Clinical Trials Area has long been interested in the laminar flow concept under development at the University of Minnesota. When it became clear that this was feasible, an effort to develop such a unit at the Clinical Center was initiated. However, because of various obstacles, there appears to be little prospect of a permanent laminar flow facility in the hospital. Accordingly, the Clinical Trials Area asked for bids for the construction of a portable laminar flow facility. A great deal of interest was engendered by the concept and a large number of organizations responded to the RFP. It is contemplated that the system will also find wide use in institutions elsewhere for patients at high risk to infection, in immunosuppression, the treatment of severe burns, etc. The contract will be awarded before the end of the fiscal year.

(3) DELINEATION OF THERAPEUTIC ACTIVITY OF AGENTS (STAGE V, STEP 2) and

(4) TUMOR ERADICATION (STAGE VI, STEP 2)

The activities which fall into this area constitute the major effort in the Clinical Trials Area. The principal task in cancer chemotherapy in addition to the identification of new agents is to delineate optimal utilization of these drugs whether it be singly or in combination with other agents in the control or eradication of cancer in man.

Leukemia

In acute lymphocytic leukemia, vincristine and prednisone have been established as the standard remission induction combination. However, because of the toxicity associated with daily prednisone, a study was done comparing four day per week prednisone with daily prednisone. There was no significant difference in the remission rate in 26 patients.

Cytosine arabinoside appears very promising in the treatment of adult AML. Complete remissions were obtained in approximately 50% of adults with AML. These results in AML are encouraging since therapeutic results in this disease have not paralleled the remarkable achievements in ALL.

The four drug combination study (prednisone, vincristine, 6-mercaptopurine, and methotrexate, POMP) has been completed. The median duration of remission after therapy was 13.5 months and the median survival from the start of therapy was almost 3 years. In adult AML the complete remission rate was approximately 50%. However, the median survival was only 6 months. Maintenance treatment and prophylactic nonabsorbable oral antibiotics did not appear to be of benefit.

In CML, it has become apparent that dibromomannitol (DBM) is a useful agent in therapy. During the year a study comparing DBM with busulfan was initiated but the data are too few to permit any conclusions at this time.

The acute phase of CML remains a difficult therapeutic problem. It was hoped that the combination of BCNU [1,3 bis-(2-chloroethyl) nitrosourea] and cytosine arabinoside would be of value. However, to the present only an infrequent patient has shown any benefit.

Preliminary results of total body irradiation of patients with chronic lymphocytic leukemia appear very promising. Good remissions are generally rare with conventional therapy but with this program approximately 45% had complete remissions. A comparative study of total body irradiation and chlorambucil is in progress.

Solid Tumors

The excellent results in therapy of Hodgkin's Disease continued during the year. The complete remission rate was more than 80% in advanced Hodgkin's Disease after treatment with a four drug combination (vincristine, procarbazine, nitrogen mustard, and prednisone). The duration of unmaintained complete remission was 18+ months (first 30 patients only). A similar study in lymphosarcoma and reticulum cell sarcoma is underway but preliminary results indicate that the regimen is not as effective.

Extended radiotherapy in advanced (Stage III) Hodgkin's Disease has been very effective with 18 of 20 patients showing complete remissions. Sixty-seven (67) patients with Stages I and II have been treated with prophylactic extended radiotherapy. All had complete remissions and to date there have been few recurrences. However, there appears to be no difference with respect to recurrence rates between conventional and extended prophylactic radiotherapy.

During the past year the Clinical Trials Area has established a Lymphoma Treatment Center at Makerere University, Kampala, Uganda (contract 67-1343), under the immediate supervision of Dr. John Ziegler. There is a large group of patients with Burkitt's tumor in the area and studies are progressing in therapy, immune host factors, and metabolism of drugs (particularly cyclophosphamide). The mean survival of 14.6 months contrasts with 2.5 months in American patients treated here at the NCI.

Studies of host factors in patients with solid tumors constitute an important activity in the Clinical Trials Area. Impairment of the immune mechanism is related to extent of involvement in Hodgkin's Disease. In contrast, there is little impairment in Burkitt's. RES clearance and bone marrow function appear to be normal in African patients with the latter disease.

Leukocyte and Tumor Kinetics

An integral part of the efforts in Stage V and Stage VI of the Chemotherapy Program Linear Array is the study of leukocyte and tumor kinetics and cell cycle characteristics. It is obvious that the most effective application of anti-tumor agents and the achievement of optimum selective toxicity depends to a large measure on knowledge of normal and tumor cell kinetics.

The effort in leukocyte kinetics which has been in progress for a number of years has yielded several important observations. The role of the spleen in leukemia has been elucidated and it appears that this organ is capable of transiently sequestering large numbers of leukemic cells. This contradicted the previous conception that the movement of cells was unidirectional from the blood to the spleen.

It has recently been shown in the first studies of leukocyte kinetics in acute leukemia in remission that the transit time and turnover rate of granulocytes in the blood is abnormal.

In vitro uptake studies of tritiated thymidine by peripheral blood leukocytes of patients with acute leukemia in remission have disclosed that the uptake is often increased over normal. The cells responsible appear to be lymphoid in origin suggesting an immune-type reaction as a consequence of the leukemic process.

Although data concerning leukocyte dynamics is accumulating relatively rapidly, knowledge in the kinetics in human solid tumors is meagre. Accordingly, during the year studies of the characteristics of neoplastic cell growth were begun. These are difficult, laborious and expensive, and to date only five patients have been studied. However, the Clinical Trials Area is in a unique position to conduct these investigations since the resources and competence are readily available here.

One of the most important obstacles to the conduct of cell kinetic studies are the difficulties in analysis of autoradiographs. A feasibility contract (Perkin-Elmer, 66-1159) for the development of an automated scanning device resulted in the development of a satisfactory scanner. The effort in automated scanning and cell recognition will continue in FY 1969 as an intramural project with the cooperation of another government agency.

To acquaint the members of the Chemotherapy Program with the latest developments in cell kinetics, a series of seminars were held and featured most of the outstanding investigators in the field. Their presentations will be published as an NCI Monograph.

(5) STUDIES ON CELL REGULATORY MECHANISMS (STAGE I, STEPS 1 and 2; STAGE V, STEP 2, ETC.)

The objectives of this activity in the Clinical Trials Area is to elucidate the control of cell growth, differentiation and carcinogenic transformation in human cells. Major emphasis is placed on regulatory mechanisms for protein synthesis (particularly t-RNA), RNA and DNA synthesis in normal and leukemic leukocytes.

There were a number of important findings and perhaps one of the most interesting was the observation that pyrimidine deoxyribosyltransferase activity in extracts from leukocytes of CML patients in remission did not return to normal. In contrast leukocytes from patients with AML in remission did return to normal. If this enzymatic deficiency is borne out in large studies of CML patients, it will be the first demonstration of a persistent biochemical defect in the morphologically normal granulocytes in CML.

Administrative Considerations

The year was an important one from an administrative standpoint with a number of difficult problems, only some of which were alleviated.

The acquisition of a 15-bed unit at the Washington Veterans Administration Hospital (transfer of funds) and the appointment of a recognized investigator (Dr. Oleg Selawry) affords the Clinical Trials Area an opportunity to conduct chemotherapeutic and kinetic studies in the more common solid tumors (lung and colon). With the limited bed capacity at the Clinical Center this is impossible.

There are serious problems and obstacles which preclude satisfactory implementation of patient isolation studies. One of the two plastic isolators was inoperative part of the year because of a nursing shortage. When this was alleviated, inability to provide adequate numbers of sterile diets prevented its use. Plans for an intensive care unit and a permanent laminar flow facility could not be implemented because space was not made available. If the intramural clinical program is to continue as a dynamic progressive activity, there will have to be an appraisal of space utilization in the Clinical Center as well as a reassessment of the relationship to the Center in general.

Clinical data retrieval is being implemented slowly but it is hoped that with FY 1969 an adequate retrieval system will be in effect.

Generally, in spite of the problems and difficulties, FY 1968 has been a busy and exciting one. Interesting and promising new agents have appeared and important information has been acquired concerning the therapeutic activity of certain agents, the supportive care of patients, and the malignant process itself.

Serial No. NCI-3406
1. Medicine Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on Cell Regulatory Mechanisms in Normal and Leukemic Leukocytes. II. Studies on the Control of Protein Synthesis at the Level of Translation of Genetic Information

Previous Serial Number: None

Principal Investigator: Robert C. Gallo

Other Investigators: Sidney Pestka and Seymour Perry

Cooperating Unit: Macromolecular Biology Section, GI&C

Man Years

Total: 2
Professional: 1
Other: 1

Project Description:

Objectives

Control of cellular growth, differentiation and carcinogenic transformation may be mediated by control of protein biosynthesis. Alterations of transfer RNA levels by cells provide a feasible means of controlling protein biosynthesis and consequently enzymatic and structural cellular components. A major purpose of this study is to determine the levels of specific transfer ribonucleic acid species in both normal and cancer cells.

Methods Employed

Normal and leukemic leukocytes from human donors are isolated by centrifugation as well as preparative column methods. Transfer RNA and aminoacyl synthetases are prepared from each purified cellular fraction by methods previously published. Where differences are localized, levels of tRNA and their coding properties will be evaluated. Computer computation of the results will be employed.

Major Findings

The methods and studies planned are being initiated at the present time.

Significance to Biomedical Research and the Program of the Institute

This study is designed to determine if there are differences between normal and cancer cells in transfer ribonucleic acid.

Proposed Course of Project

The course of the project will be determined after the initial studies are completed. We plan to evaluate the effects of various therapeutic agents on the aminoacyl-tRNA levels in cancer cells and to correlate the results with therapeutic response.

Honors and Awards

None.

Publications

None.

Serial No. NCI-3409
1. Medicine Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Study of Leukocyte Kinetics in Normal Individuals and in Patients with Leukemia

Previous Serial Number: SAME

Principal Investigator: Seymour Perry

Other Investigators: Jerry L. Spivak, Leonard H. Brubaker and John J. Gart

Cooperating Unit: Biometry Branch, NCI

Man Years

Total: 5
Professional: 3
Other: 2

Project Description:

Objectives

1. To investigate granulocyte kinetics in acute leukemia before and after therapy.

2. To define mechanisms of Cr^{51} labeling of leukocytes.

3. To investigate the leukokinetic patterns in chronic lymphocytic leukemia for both lymphocytes and granulocytes using Cr^{51} and DFP^{32}.

4. To investigate the leukocyte release mechanism from the bone marrow utilizing etiocholanolone.

5. To study peripheral blood leukocyte kinetics in patients with leukemia utilizing tritiated thymidine.

6. To evaluate the significance of increased DNA synthesis by circulating leukocytes of patients with acute leukemia in remission.

Methods Employed

1. In vitro labeling of blood granulocytes was accomplished by labeling whole blood in vitro with DFP^{32}. After infusion, specific activity of the isolated leukocytes was monitored by liquid scintillation counting.

2. Factors affecting labeling by Cr^{51} of blood cells were studied using red blood cells and leukocytes from patients with chronic lymphocytic leukemia and acute and chronic myelocytic leukemia. Cell homogenates were also utilized to study the characteristics of intracellular binding.

3. Double label procedures were used to study kinetics of lymphocytes and granulocytes labeled simultaneously with Cr^{51} and DFP^{32} respectively. A one inch collimated crystal was used to scan for white cell radioactivity over body surfaces.

4. A study was instituted in normal volunteers using Cr^{51} and DFP^{32} as a double label for granulocytes. The objective here was to study Cr^{51} elution and the effect of Cr^{51} on the granulocyte life span.

5. In vitro uptake of tritiated thymidine by isolated peripheral leukocytes was accomplished with a short term tissue culture technique followed by isolation of the DNA for liquid scintillation counting. Radioautographs were made for identification of labeled cells.

Major Findings

1. Acute leukemia: These are the first studies of granulocyte kinetics in acute leukemia in remission. The half disappearance time of granulocytes is prolonged in acute leukemia both in remission and in relapse. The turnover rate is decreased and the granulocyte disappearance curve is markedly different from normal.

2. Mechanism of Cr^{51} labeling of leukocytes: Cr^{51} enters leukocytes in the hexavalent cationic but not in the trivalent anionic state. The Q10 is 1.2. Uptake is greater at 37°C than 24°C than 4°C. Only 3% of the label is taken up and the rate of uptake is greater per 10^7 white cells than for red cells. Some intracellular Cr^{51} is protein bound and trivalent (extent to be determined). Cr^{51} in amounts greater than 10 $\mu c/10^8$ cells affects cell respiration. The double label studies have also shown that Cr^{51} does not appreciably elute from white blood cells as it does from red cells.

3. Leukokinetic patterns in chronic lymphocytic leukemia: CLL lymphocytes labeled with Cr^{51} show a lymphocyte survival curve which is not affected by splenomegaly or the height of the white blood count. The first phase is short with a variable T 1/2; the second T 1/2 between 92 and 126 hours. The data fit a two compartment model. Lymphocytes pool in the spleen and are only transiently trapped in the lung. There appears to be recycling of lymphocytes through the spleen. Granulocyte curves in these patients are abnormal unless they are in remission. The T 1/2 correlates with the size of the spleen.

Double label studies in patients with carcinoma of the lung reveal an abnormal DFP^{32} curve not reflected by the Cr^{51} labeled cells. Patients with Hodgkin's disease also show abnormal granulocyte survival curves. Both of these findings have not been previously noted.

4. A survey of patients with acute leukemia has disclosed that the uptake of tritiated thymidine in peripheral leukocytes by the method described is elevated in patients having circulating "blast" cells. The thymidine uptake is markedly increased following chemotherapy and reaches highest values around the time of early remission. During the first month of remission, the value often falls to normal levels where it remains until a relapse again produces circulating "blast" cells. However, in some patients, the thymidine uptake remains elevated or fluctuates between elevated and normal values. The predominant cell types found to be labeled during remission are large mononuclear cells having light to basophilic cytoplasm, similar to those previously described in normals, in patients with autoimmune disease, aplastic anemia, etc.

Particular emphasis is being given to patients with acute myelogenous leukemia in whom the presence of increased numbers of lymphoid cells could not be attributed directly to the leukemia. There is a possibility that the increased thymidine uptake in early remission is related to an immune-type reaction against leukemic cell antigens similar to that postulated by others in Hodgkin's disease.

Honors and Awards:

Dr. Seymour Perry

1. Coauthor of chapter in a book entitled "Regulation of Hematopoiesis" edited by Dr. A. S. Gordon. Title of chapter: "Physiology of Human Normal and Leukemic Leukocytes: Kinetics and Biochemical Considerations."

2. The Study of Leukocyte Dynamics in Chronic Lymphocytic Leukemia, and Lymphocyte Recirculation in Normal Man, presented at the meetings of The French Society of Hematologists, France, 1968.

Publications:

1. Wolff, S. M., Kimball, H. R., Perry, S., Root, R., and Kappas, A.: The biological properties of etiocholanolone. Ann. Int. Med. 67: 1268-1295, Dec., 1967.

2. Vogel, J. M., Kimball, H. R., Wolff, S. M., and Perry, S.: Etiocholanolone in the evaluation of marrow reserves in patients receiving cytotoxic agents. Ann. Int. Med. 67: 1226-1238, Dec., 1967.

3. Perry, S., Godwin, H. A., and Zimmerman, T. S.: Physiology of the granulocyte. J.A.M.A. (Part I) 203: 937-944; (Part II) 203: 1025-1032, March 1968.

4. Godwin, H. A., Zimmerman, T. S., Kimball, H. R., Wolff, S. M., and Perry, S.: Correlation of granulocyte mobilization with etiocholanolone and the subsequent development of myelosuppression in patients with acute leukemia receiving therapy. Blood (in press).

5. Godwin, H. A., Zimmerman, T. S., Kimball, H. R., Wolff, S. M., and Perry, S.: The effect of etiocholanolone on the entry of granulocytes into the peripheral blood. Blood (in press).

6. Godwin, H. A., Zimmerman, T. S., and Perry, S.: Peripheral leukocyte kinetic studies of acute leukemia in relapse and remission and chronic myelocytic leukemia in blastic crisis. Blood (in press).

7. Zimmerman, T. S., Godwin, H. A., and Perry, S.: Studies of leukocyte kinetics in chronic lymphocytic leukemia. Blood 31: 277-291, March 1968.

8. Zimmerman, T. S., Godwin, H. A., Zelen, M., and Perry, S.: Remission and relapse status of acute leukemia as studied by the in vitro uptake of tritiated thymidine by peripheral blood leukocytes. Blood (in press).

9. Duvall, C. P., and Perry, S.: The use of 51-chromium in the study of leukocyte kinetics in chronic myelocytic leukemia. J. Lab. Clin. Med. 71: 614-628, April 1968.

10. Perry, S.: Leukocyte Kinetics in Leukemia. International Conference on Leukemia-Lymphoma Monograph, University of Michigan (in press).

Serial No. NCI-3411
1. Medicine Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on Cell Regulatory Mechanisms - Normal and Leukemic.
I. Control of Pools of Deoxynucleoside DNA Precursors

Previous Serial Number: Same

Principal Investigator: Robert C. Gallo

Other Investigators: Seymour Perry, Jacqueline Whang-Peng and
T. R. Breitman

Cooperating Units: Laboratory of Physiology, NCI

Man Years:

Total: 2 1/2
Professional: 1 1/2
Other: 1

Project Description:

Objectives

1. To elucidate the precise enzymatic mechanisms for the "salvage pathway" of DNA synthesis in human leukocytes.

2. To study the control mechanisms for the enzymatic synthesis of deoxythymidine in these cells, and to quantitate differences between normal and leukemic cells.

3. To study regulatory mechanisms for DNA, RNA, and protein synthesis in intact normal and leukemic leukocytes.

Methods Employed

1. Leukocytes were isolated from patients with chronic myelogenous leukemia by a dextran sedimentation hypotonic shock method previously developed in this laboratory. Standard procedures were modified 1) for incubation of leukocytes, 2) cell-free incubations and identification of reaction products, 3) isolation of DNA, RNA, and proteins, and 4) for purification of enzymes by standard biochemical techniques.

2. The kinetics of deoxythymidine synthesis was studied in cell-free systems using homogenized, normal, and leukemic leukocytes.

3. The effect of phytohemagglutinin on deoxythymidine synthesis is under study.

Major Findings

1. Both leukemic and normal leukocytes synthesize deoxythymidine by two enzymatic mechanisms: a) direct transfer of the deoxyribosyl moiety to thymine from a deoxynucleoside donor and b) by a coupled deoxynucleoside phosphorylase in which the deoxynucleoside is first cleaved to deoxyribose 1-phosphate and the pyrimidine base. The deoxyribose 1-phosphate then reacts with thymine to synthesize deoxythymidine.

2. There is relative specificity of the pyrimidine deoxynucleosides for the first mechanism (direct deoxyribosyl transfer) and by the purine deoxynucleosides for the second (coupled). This was found with both normal or leukemic leukocytes as the enzyme source.

3. Deoxythymidine synthesis by either mechanisms was greatest with enzyme preparations obtained from normal (60-85%) granulocytes. It was least with enzyme preparations obtained from lymphocytes (normal or leukemic). Leukocytes from patients with chronic myelogenous leukemia (CML) had intermediate values (approximately 1/2 the value of normal granulocytes). This was true even when purine deoxynucleosides (deoxyadenosine, deoxyinosine, or deoxyguanosine) served as the deoxyribosyl donor despite the fact that purine deoxynucleosides take part in deoxythymidine synthesis by a coupled mechanism (phosphorylase). Determination of rates of purine deoxynucleoside cleavage by a phosphorolytic mechanism was as great or greater when CML cells were the source of the enzyme preparation.

4. The synthesis of deoxythymidine was much greater (20-30 fold) when a pyrimidine deoxynucleoside was the deoxyribosyl donor than when a purine deoxynucleoside was the donor. This ratio was held for both normal and leukemic leukocytes.

5. An attempt was made to determine the reason for the limited support of deoxythymidine synthesis by purine deoxynucleosides despite the fact that cleavage of these deoxynucleosides (and therefore production of deoxyribose 1-phosphate) appeared more than sufficient. It was speculated that a product of purine cleavage might inhibit deoxythymidine synthesis from thymine and deoxyribose 1-phosphate. We have found that the purine bases, hypoxanthine, xanthine, guanine, and adenine all inhibit deoxythymidine synthesis.

6. The kinetics of inhibition of deoxythymidine synthesis is very complex, but appears to have at least partly overcome by increasing concentrations of deoxyribose 1-phosphate.

7. Because of their clinical importance, certain synthetic analogues of the naturally occurring purine bases were also tested for their effects on deoxythymidine synthesis. Allopurinol inhibits deoxythymidine synthesis (the

magnitude of inhibition is comparable to hypoxanthine and xanthine). The greatest inhibition is produced by 6-mercaptopurine.

8. The kinetics of inhibition by 6-MP and allopurinol appears to be the same as the kinetics of inhibition for the naturally occurring purine bases.

9. In the course of these studies on the kinetics of deoxythymidine synthesis, we have found that a precise concentration of thymidine is critical in determining the rate of deoxythymidine synthesis. Above a concentration of 2.5 mM, thymine inhibits deoxythymidine synthesis (substrate inhibition). Since it has been previously shown by Freidkin that thymine inhibits deoxythymidine breakdown at concentrations of approximately 0.5 to 1.0 mM, precise concentrations of thymine may be important in the regulation of the salvage pathway for DNA synthesis, i.e., concentrations of approximately 1.0 mM may increase the deoxythymidine pool by inhibiting deoxythymidine cleavage while synthesizing deoxythymidine. Concentrations above 2.5 mM would inhibit further deoxythymidine formation.

10. The kinetics of substrate inhibition by thymine revealed that thymine competes for the deoxyribosyl donor site when the donor is a deoxynucleoside. When the donor is deoxyribose 1-phosphate, the kinetics were more complex.

11. Phosphate and arsenate were found to be activators of pyrimidine deoxyribosyl transferase.

12. Pyrimidine deoxyribosyltransferase activity of extracts obtained from leukocytes of patients (about 30 assays) with chronic myelogenous leukemia (CML) was approximately one-half the activity found in normal leukocyte extracts. The deficiency remained even when the patients were in complete hematologic remission. However, a detailed kinetic analysis of the enzyme revealed no detectable qualitative difference between the CML and normal enzyme. The deficiency in the transferase, therefore, appears to be the result of an actual decrease in amount of enzyme present.

13. Phytohemagglutinin increases the activity of thymidine phosphorylase. The increase in activity does not appear to be due to an increase in the _de novo_ production of the enzyme.

Honors and Awards

Dr. Robert C. Gallo

1. Invited to lecture at the Department of Biochemistry, Ohio State University, by Dr. David Ives on "Enzymatic Mechanisms for Deoxythymidine Synthesis."

2. Invited to co-author a chapter for inclusion in a monograph entitled "Regulation of Hematopoiesis" edited by Dr. A. S. Gordon. Title of chapter will be "Physiology of Human Normal and Leukemic Leukocytes:

Honors and Awards (continued)

Kinetics and Biochemical Considerations."

3. Invited to lecture at Georgetown University, Department of Medicine, Washington, D. C.

4. Invited to lecture at Jefferson Medical College, Philadelphia, Pennsylvania, by Dr. A. J. Erslev.

5. Invited to present seminar at the U.S. Public Health Service Hospital, Baltimore, Maryland.

6. Invited to present seminar to the staff of Section of Human Biochemical Genetics, NIAMD, NIH.

7. Invited to present seminar to staff of the Laboratory of Physiology, NCI.

Publications

1. Gallo, R. C., Perry, S., and Breitman, T. R.: The enzymatic mechanisms for deoxythymidine synthesis in human leukocytes. J. Biol. Chem. 242: 5059-5068, Nov. 1967.

2. Gallo, R. C., and Breitman, T. R.: The enzymatic mechanisms for deoxythymidine synthesis in human leukocytes. II. Comparison of various deoxyribosyl donors. J. Biol. Chem. (in press).

3. Gallo, R. C., and Breitman, T. R.: The enzymatic mechanisms for deoxythymidine synthesis in human leukocytes. III. Inhibition by purines. J. Biol. Chem. (in press).

4. Gallo, R. C., and Perry, S.: Deficiency of leukocyte pyrimidine deoxyribosyltransferase: An enzyme abnormality in human leukaemia. Nature (in press).

5. Gallo, R. C., and Perry, S.: Inhibition of human leukocyte pyrimidine deoxynucleoside synthesis by allopurinol and 6-mercaptopurine. Biochem. Pharmacol. (in press).

Serial No. NCI-3412
1. Medicine Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on the Role of Viruses in the Etiology of Malignant Diseases

Principal Investigator: Yashar Hirshaut

Other Investigators: Ronald Yankee and Seymour Perry

Cooperating Units: Viral Biology Branch, NCI, Viral Leukemia and Lymphoma Branch, NCI

Man Years:

Total: 1
Professional: 2/3
Other: 1/3

Project Description:

Objectives

1. To detect new viral agents in tissue specimens taken from patients with leukemia, lymphoma, Hodgkin's disease and a variety of sarcomas.

2. To define methodology of virus isolation from human sources.

3. To assist the Viral Biology Branch in determining whether virus-like particles found in human serum are of pathogenic significance.

4. To assist the Viral Leukemia and Lymphoma Branch in determining whether an "incomplete" virus could be isolated from human sarcoma tissues using material from leukemic patients as a source of "helper" virus.

Major Findings

Virus-like particles of "C" type morphology were found in the sera of about 20% of patients with Hodgkin's disease. These were present in serum pellets examined by electron microscopy. In no instance was virus found to be budding from a sellular surface. The significance of their presence is difficult to determine since these particles are morphologically similar to other membranous fragments of various sizes and shapes that are though to represent typical cellular debris.

Twenty liters of human leukemic sera which were negative for "C" type virus-like particles were sent to the John L. Smith Laboratory of the Pfizer Company for ultracentrifugation and preparation of "virus" pellets. This material was packaged and distributed for use in "helper" virus studies.

The effort to detect "C" type particles in the sera of additional leukemic patients has been discontinued temporarily until more definitive methods become available to determine whether such morphologic entities possess biologic activity. In vivo transmission studies with monkeys and chimpanzees have been undertaken with material already available but none of the inoculated animals have developed a lymphoproliferative disorder, to date.

Ten tissue specimens of sarcomatous tissue have been submitted to study participants in an effort to isolate tissue culture lines. These attempts have yielded four long-term cultures. When murine leukemia virus and human leukemic serum pellets were added to these cultures no new "incomplete" virus or viral-like activity could be demonstrated.

Significance

As animal studies continue to reveal that viruses of "C" type morphology are responsible for leukemia in more and more species there is growing interest in determining whether human leukemia is also caused by a similar agent. Similarly, as more is learned about the relationship between animal leukemia and sarcoma viruses, it becomes intriguing to consider whether such a relationship can be demonstrated in humans as well. So far, however, the application of animal techniques to human material has not succeeded in producing convincing evidence that viruses and cancer are related in any way. Further work utilizing newer tissue culture methods, and newborn animal models may eventually provide the key to progress in this area.

Publications:

None

Serial No. NCI-3414
1. Medicine Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Cytogenetic Studies in Patients with Neoplastic Disease and the Effect of Drugs upon Chromosomes

Previous Serial Number: SAME

Principal Investigator: Jacqueline Whang-Peng

Other Investigators: Paul P. Carbone, Joe Hin Tjio, Seymour Perry, Edward Henderson, Sarah Stewart, C. J. Dawe, John Bennett, Brigid Leventhal and Lawrence Chessin

Cooperating Units: Laboratory of Pathology, NCI, Viral Biology Branch, NCI, and Laboratory of Pathology, LCI

Man Years:

Total:	5
Professional:	3
Other:	2

Project Description:

Objectives

1. Cytogenetic studies in patients with leukemia.

2. The effect of therapeutic agents on chromosomes.

3. Lymphocyte transformation studies.

Methods Employed

Standard cytogenetic techniques were used except as specified.

Major Findings

1. Cytogenetic studies in CML - see Project No. NCI-3404.

2. Continuous cell culture line from a hepatocarcinoma induced in a rhesus monkey by N-nitro-diethylamine.

Both the E and F sublines are subtriploid, and have a large percentage of cells with 2 marker chromosomes: 1 large metacentric and 1 small acrocentric.

However, the modal number of the F subline is 56, while that of the E subline is 59. The E subline is characterized by trisomy of chromosome number 20, which is disomic in subline F.

3. Continuous cell culture lines from patients with infectious mononucleosis, all had normal diploid karyotypes.

4. Daunomycin. Cytogenetic, morphologic, and biochemical abnormalities in leukocytes from 7 patients with acute lymphocytic or granulocytic leukemia were studied after in vivo and in vitro exposure to daunomycin. The highest percentage of major chromosome aberrations (80-90%) appeared immediately after treatment. These aberrations consisted of chromatid breaks, fragments, chromatid exchanges, ring chromosomes, dicentrics or extensive fragments and disappeared within one or two weeks. The in vitro studies on human lymphocytes indicate that daunomycin affects DNA synthesis more than RNA synthesis, and that it also interferes with the cell cycle during G2 and delays mitosis in cells which have already synthesized DNA.

5. LSD was tested in human, mouse and rabbit lymphocytes. The in vitro dose for human peripheral blood lymphocytes was 1×10^{-3}, 1×10^{-2}, 1 and 10 gamma per cc of medium. There were no abnormalities detected in the bone marrows, spleens and peripheral blood from the animals immediately after administration. With the 5 different concentrations of LSD mentioned above, when added at time zero to normal peripheral lymphocytes, the incidence of chromosomal aberrations (breaks, fragments, chromatid exchanges, rare ring and dicentrics) was approximately the same. Minimal aberrations were observed if the drug was added after protein synthesis had been completed (i.e., 6 hours).

6. In vitro study of blast crisis stage of CML peripheral blood cells.

H^3TdR as a pulse label was used to study the generation time, maturation and phagocytosis by CML leukocytes.

From the data so far, some of the blast cells may undergo maturation in 24 hours, but others never mature, remaining either in the S or G2 period of the mitotic cycle. The observation that precursor cells are capable of phagocytizing both bacteria and polystyrene particles may be a consequence of maturation. More of the cytoplasm may have matured although nuclear maturation had not yet occurred.

The study of factors responsible for this maturation in culture are in progress.

Publications:

1. Whang-Peng, J., Perry, S., and Knutsen, T.: Maturation and Phagocytosis by Chronic Myelogenous Leukemia Cells In Vitro: A Preliminary Report. J. Nat. Cancer Inst. 38: 969-977 (June), 1967.

2. Whang-Peng, J., Tjio, J. H., and Cason, J. C.: Reutilization of Presumably Degraded Radioactive Label from a Chromosomal Fraction of Homologous and Heterologous Cells. Proc. Soc. Exp. Biol. Med. 125: 260-263 (July) 1967.

3. Glade, P. R., Kasel, J. A., Moses, H. L., Whang-Peng, J., Hoffman, P. F., Kammermeyer, J. K., and Chessin, L. N.: Infectious Mononucleosis: Continuous Suspension Culture of Peripheral Blood Culture. Nature 217: 564-565 (Feb.) 1968.

4. Dawe, C. J., Whang-Peng, J., Morgan, W. D., O'Gara, R. W., and Kelly, M. G.: Culture of a Cell Line (NCLP-6) Derived from a Hepatocarcinoma Induced in Macaca Mulatta by N-nitrose-diethylamine. J. Nat. Cancer Inst. (in press).

5. Whang-Peng, J. and Bennett, J. M.: Cytogenetic Studies in Metastatic Neuroblastoma. Am. J. Dis. Child. (in press)

Serial No. NCI-3415
1. Medicine Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: NCI-IBM Blood Cell Separator

Previous Serial Number: NCI-3415

Principal Investigators: C. Dean Buckner, Robert Graw, Robert Ralph, Edward Henderson and Seymour Perry.

Other Investigators: Robert Eisel, Coralyn Jones and Jacqueline Whang-Peng

Man Years

Total:	6
Professional:	3½
Other:	2½

Objectives:

1. To develop methods of buffy coat collections in man utilizing the NCI-IBM blood cell separator.

2. To determine the effects of buffy coat infusions in patients with leukemia and other malignancies.

Methods Employed

The NCI-IBM blood cell separator has been run 98 times in man for periods of 1-6 hours for collection of buffy coat cells. Ninety-two of the centrifugations were performed on twelve patients with CML. Four patients who were normal hematologically also underwent centrifugation and cell collection: 2 patients with carcinoma underwent one centrifugation each, 2 normal donors underwent four centrifugations. In all these procedures, a vein to vein technique was utilized. In addition, one patient underwent three plasma exchanges for hepatic coma.

The effects of centrifugation on blood elements were studied by routine hematological tests and infusion into leukopenic and non-leukopenic recipients.

Major Findings

1. Buffy coat cells from CML donors can be collected in a small volume with a high or low platelet count and a high white blood cell count. In an average CML donor with a WBC of 70,000 per mm^3, approximately

Major Findings (continued)

1×10^{11} leukocytes per hour can be collected.

2. In normal donors, buffy coat with a preponderance of lymphocytes or granulocytes can be collected without production of thrombocytopenia in the donor.

3. Transfused CML cells produce increments in leukemic patients that are compatible on a dose basis to increments obtained by transfusion of CML cells processed by routine plasmapheresis. Ninty-six infusions have been performed in fifteen separate patients.

4. One normal donor was centrifuged for the collection of granulocytes and 20×10^9 cells were obtained. These cells produced an increment of $900/\text{mm}^3$ in a granulocytopenic patient.

5. One normal donor was centrifuged three separate times for the collection of lymphocytes without significant red cell contamination.

6. One patient with acute hepatic necrosis underwent three plasma exchanges of 5, 8, and 6 liters. This was technically a successful procedure and bilirubin levels were dramatically lowered without significant removal of red blood cells.

Part B Honors, Awards, and Publications

1. Buckner, D., Eisel, R., and Perry, S.: Blood Cell Separation in the Dog in Continuous Flow Centrifugation. Blood (in press).

2. Judson, G., Kellogg, R., Jones, A., Buckner, D., Eisel, R., Perry, S., and Greenough, W.: A Continuous Flow Centrifuge. Nature April 1968.

Serial No. NCI-3400
1. Medicine Branch
2. Leukemia Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Part A

Project Title: The Chemotherapy of Acute Leukemia

Principal Investigator: Edward S. Henderson

Other Investigators: Brigid Leventhal, Patrick Henry, Ronald Yankee and Arthur Serpick, Roland Skeel

Cooperating Units: Cancer Chemotherapy National Service Center
Laboratory of Chemical Pharmacology
Leukemia Cooperative Group B, James F. Holland, Chairman
U.S. Public Health Service Hospital, Baltimore, Maryland

Man Years (July 1, 1967 to June 30, 1968):

Total:	3	
Professional:	2	(does not include Clinical Associates)
Other:	1	

Project Description:

Objectives:

1. To identify new chemotherapeutic agents active against acute leukemia.

2. To improve the effectiveness of compounds with known activity against acute leukemia.

3. To develop efficient experimental designs for the collection of quantitative chemotherapy data.

4. To relate the effects in man to pre-clinical studies of pharmacology, experimental therapeutics, and animal tumor biology, in order to develop effective predicting systems for development of new agents.

Major Findings:

Three major tasks of the chemotherapist in developing more effective anti-leukemic regimens are: 1) identifying new agents, 2) perfecting the applicating of these drugs in the light of knowledge of their pharmacological disposition and the kinetics of their cytotoxic activity, and 3) combining their activities so as to increase their therapeutic effectiveness. During FY 1968 the Leukemia Service has addressed its attention to these three areas as follows:

I. New Drug Activities:

Studies are currently in progress to evaluate NSC 82196, imidazole carboximide bis-β-chlorethyl triazeno, a compound with high activity against murine leukemia L1210, plus L-asparaginase which has to date shown activity against "spontaneous" leukemia in many mammalian species including man. To date three patients with acute lymphocytic leukemia have received once weekly injections of 400 mg/M^2 of NSC 82196 without therapeutic effect. Of four patients receiving L-asparaginase, one adult and one child with acute lymphocytic leukemia have responded to date. Both studies are preliminary and will be extended.

II. Improved Drug Activities:

Cytosine Arabinoside - Based on the premise that the effect of drugs affecting cells in replication depends upon the product of the concentration of the drug and the duration of cell exposure (C x T), cytosine arabinoside was given in daily infusions of four hours duration for four day courses to 40 patients with acute myelocytic leukemia. Each infusion was preceded by a priming dose to produce an effective plasma level, and this level was maintained by drug infusions tailored to the known plasma disappearance rate of cytosine arabinoside. Sixty per cent of the patients responded (47.5% complete remissions, 12.5% partial remissions). This schedule doubled the effectiveness of the drug as compared to its use by daily rapid injection or unprimed 4-24 hour infusion. The increased effectiveness was demonstrated despite a mortality of only 12 per cent, a remarkably low figure for any therapy of acute myelocytic leukemia.

Vincristine plus Prednisone - The combination of weekly vincristine plus daily prednisone is a standard remission induction combination for acute lymphocytic leukemia. Daily prednisone imposes significant toxicity in the form of fluid retention, hypertension, etc. Studies with non-neoplastic disease (e.g., rheumatoid arthritis and nephrosis) have demonstrated that four day courses of steroids given once weekly result in less morbidity and less perturbation of the pituitary-adrenocortical axis. A comparison of weekly vincristine combined with either daily or four day/week prednisone demonstrated no significant difference in rate of remission in 26 patients with acute lymphocytic leukemia.

III. Combination Chemotherapy

POMP - The evaluation of the four drug combination of prednisone, vincristine, 6-mercaptopurine, and methotrexate in acute leukemia is essentially complete. In 35 children with acute lymphocytic leukemia, 32 achieved a first remission, and 90 per cent of those who relapsed were reinduced with the same four drugs. Median duration of remission (13.5 months), median duration of unmaintained remission, the 16 patients who completed all therapy (8 months), and median survival from the start of treatment to death (32 months) have been established. No significant change in the rate of relapse was seen when therapy was terminated, thus indicating that for this group the course of the disease was determined by the interaction a) the intrinsic pathophysiology of each patient's leukemic cell population and b) the effects of early therapy (i.e., treatment given during the first 2-4 months of the illness.

The results in children with acute myelocytic leukemia, and adults with both acute lymphocytic and acute myelocytic leukemia respectively are tabulated below:

	# Pts.	% Complete Remissions	Median Duration of Remission (months)	Median Duration Survival (months)
Childhood AML	11	81	7	18
Adult AML	24	46	7	6
Adult ALL	11	81	4	12

Comparable figures for the enlarged protocol 6513A conducted in collaboration with Acute Leukemia Cooperative Group B are:

Adult AML	87	29	4	Not Completed
Adult ALL	24	50	6	Not Completed

Maintenance treatment during remission was not found to be of value in either groups, nor did non-absorbable oral antibiotic prophylaxis result in increased responsiveness or survival.

A second ALCG-B study to compare the effectiveness of vincristine plus prednisone versus POMP against adult acute lymphocytic leukemia is in progress. To date 5 of 6 patients on POMP as opposed to 3 of 6 on vincristine and prednisone have responded. The unresponsive patients all died. Although the number of patients is small, POMP appears to provide better control of the form of adult leukemia.

COMP - The POMP combination, although very successful, has the practical restriction of combining two potent hepatic toxins (6-mercaptopurine and methotrexate) and the theoretical limitation of relying chiefly on cell-cycle dependent drugs (6-MP, methotrexate, and vincristine). It also follows an arbitrary once per month maintenance schedule, which may or may not be appropriate for the individual patient. The COMP combination was designed to remedy and/or evaluate these factors. Children with acute lymphocytic leukemia are put into remission with vincristine and prednisone following which courses of vincristine, prednisone, methotrexate and cyclophosphamide are administered. Cyclophosphamide is an alkylating agent whose activity is not restricted to cells in DNA synthesis. It also has relatively little hepatotoxicity. Patients were randomly assigned to either a fixed monthly maintenance schedule or to a schedule determined by the status of granulocyte reserve of each patient, determined by the etiocholanolone stimulation test. At present neither schedule appears to be superior to the antecedent POMP program. Cyclophosphamide alone is a less effective drug than 6-MP alone in terms of remission maintenance and the combination of cycle dependent and non-cycle dependent agents does not appear to overcome this difference.

Significance:

As stated in last year's annual report, early intensive chemotherapy appears to provide optimal control of acute leukemia in both adult and childhood groups. The objectives of chemotherapy research remains unchanged: the identification of new compounds, especially those with mechanisms of action distinct from those currently available, and the refinement of existing activities so as to provide maximum effectiveness at minimum cost in toxicity. Such studies properly conducted require much time (e.g., 4 years for the completion of the POMP study) and the highest level of support in terms of blood cell replacement, antibiotic research, and reverse isolation facilities. A further desideration is the development of quantitative techniques to gauge tumor and normal stem cell responses and kinetics so that any treatment can be optimally individualized for each patient.

Proposed Course:

Future chemotherapy studies shall include:

1. Studies of the remission maintenance capability and schedule dependency of L-asparaginase.

2. The effect of high dose prednisolone and, when available, L-asparaginase in eliminating residual leukemia foci in patients maximally treated with a POMP-type regimen.

3. Comparison of POMP versus a combination of vincristine, prednisone and daunomycin in remission induction of adult acute lymphocytic leukemia.

4. Combinations of cytosine arabinoside infusions with other drugs effective against acute myelocytic leukemia (e.g., daunomycin, 6-MP and 6 methyl mercaptopurine riboside) for induction and maintenance of this disease.

5. Initial clinical trials of new agents, e.g., NSC 82196; chlorocyclohexyl nitrosourea; Trifluoro thymidine; and streptozotocin.

6. Continued analysis of indicators of residual disease and responsiveness.

Part B Honors, Awards, and Publications

1. Henderson, E.S. The rationale for combination chemotherapy of the acute lymphocytic leukemia of childhood. Proceedings of the Fifth International Congress of Chemotherapy. 1967.

2. Henderson, E.S., Freireich, E. J, Henry, P.H., and Leventhal, B.G. Combination chemotherapy in acute lymphocytic leukemia of childhood. Proc. of the Fifth International Congress of Chemotherapy. 129-137, 1967.

3. Henderson, E. Combination Chemotherapy of Acute Lymphocytic Leukemia of Childhood. Cancer Research 27: 2570-2572, Dec. 1967.

4. Serpick, A.S. and Henderson, E.S. Observations on toxicity and clinical trials with daunomycin. Pathologie et Biologie, 15: 909-912, 1967.

5. Skeel, R. T., Henderson, E. S., and Bennett, J. M. The Significance of Bone Marrow Lymphocytosis of Acute Leukemia Patients in Remission. Blood. In Press

Publications (cont'd)

6. Yankee, R. A., DeVita, V. T., and Perry, S. The Cell Cycle of Leukemia L1210 Cells _In_ _Vivo_. Cancer Research, 27:2381-2385, 1967.

Serial No. NCI-3401
1. Medicine Branch
2. Leukemia Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968
Formerly NCI 3401 and NCI 3413

Part A

Project Title: Clinical Complications of Acute Leukemia and Their Management

Principal Investigator: Edward S. Henderson

Other Investigators: Ronald A. Yankee, Brigid Leventhal, John Hazen, Harvey Preisler, Roland Skeel, Ante Lundberg, Carl Leventhal, and Seymour Perry

Cooperating Units: Blood Bank, Clinical Center
Ophthalmological Branch, NINDB
James F. Mitchell Foundation, Washington, D.C.
American National Red Cross, Washington, D.C.
Children's Hospital of Philadelphia, Philadelphia, Pa.
Children's Cancer Research Foundation, Boston, Mass.
Travenol Division, Baxter Laboratories, Morton Grove, Illinois
Laboratory of Chemical Pharmacology, C., NCI
Program Analysis Branch, NCI

Man Years

Total:	11
Professional:	5
Other:	6

Project Description:

Objectives:

1. To protect patients with drug and/or disease induced granulocytopenia by fresh and frozen leukocyte transfusions, germ free environment, and stem cell grafting.

2. Evaluate the long term effects of intrathecal antifol therapy on CNS function and histology.

3. Assess fresh and frozen platelets in the prevention and control of thrombocytopenia induced hemorrhage.

Objectives: (continued)

4. Assess effects of chemotherapeutic agents on renal, hepatic, endocrine, neurological, etc. functions.

5. Assess extent of and methods for diagnosing and treating microbial infections as a complication of chemotherapy.

6. Complications of the natural course of leukemia.

Major Findings:

1. Leukocyte transfusions. Leukocytes for transfusion were obtained primarily from the NCI-IBM separator. (See NCI-IBM project report). Comparative transfusion studies including increments/$M^2/10^{11}$ cells and leukocyte survival in recipients were similar to that obtained by plasmapheresis. Higher actual increments in machine isolated leukocytes reflect higher dose levels obtained by this method over plasmapheresis. Pseudomonas septicemia in acute leukemia patients from 1961 to 1967 were reviewed with respect to survival related to the use of leukocyte supportive therapy. Eleven of 17 patients who received no leukocytes died within 10 days (65%) as compared with 21 of 38 patients (55%) who received leukocytes. Low morbidity directly attributable to leukocyte transfusions was observed. Sixty-six per cent of patients receiving leukocytes lived longer than one month.

   Leukocyte preservation is in progress. Large quantities of cells from CML donors are being preserved in liquid nitrogen for future autologous transfusion during blast crisis. Frozen leukocytes have not as yet been transfused.

2. "Life Island"reverse isolation. This study was handicapped by a lack of dietitians to provide adequate numbers of sterile diets for the contemplated studies. Only one reverse isolation unit could be operated during the majority of FY 1968. Despite this 5 patients with bone marrow hypofunction (2 with aplastic anemia, 1 choriocarcinoma and 2 with acute leukemia) spent an average (mean) of 61 days in the sterile environment. This brings to 21 the total occupancy on the "Life Island" since its introduction September 1964. The total patient days to date stands at 1075, the mean duration of isolation at 51.2 days with a range of 4-111. As a parallel control four patients have been placed on a sterile food and oral antibiotic regimen identical to that received by the reverse isolation group, and 3 additional patients have been placed in a routine ward care group. These randomly assigned patients ("Life Island",sterile food non-isolated, and regular ward groups) will be compared for incidence of infection, response to treatment, and serial bacterial evaluation on a prospective basis. Of the 5 isolated patients, the female choriocarcinoma patient, refractory to chemotherapy on the open ward, achieved a complete clinical and biological remission (normal

Major Findings: (continued)

chorion gonadotrophin titers) for at least 7 months, one acute leukemic responded to cytosine arabinoside therapy after 90 infection free days in the "Life Island." The other acute leukemic died after an unsuccessful attempt at allogeneic marrow grafting. Of the 2 aplastic anemia patients one died of an acute cerebral accident, but the second has returned to full activity with a steadily improving bone marrow.

3. Platelet transfusions. Clinical trials with acidified platelet concentrates and non-acidified platelet concentrates were carried out. Results show that when non-acidified platelets are centrifuged at 14-22°C and allowed to stand for 1 hour after removal of plasma the lack of clumping during resuspension of the concentrate compares favorably with immediate resuspension of acidified concentrates. The per cent recovery of the then infused platelets at one hour are exactly the same for both methods. Platelet preservation studies have continued in collaboration with the American National Red Cross. Cryopreservation of platelets using DMSO has been further evaluated. The 50 per cent recovery of frozen platelets compared with fresh acid concentrates was similar whether cells were frozen in 6.5% DMSO or 6.5% DMSO plus 5% glucose. Variation in the rate and control of freezing between 1-5°C/min. did not appear to affect the one hour recovery of transfused platelets after thawing and washing. A platelet computer program was formulated and is presently being used for rapid retreival of data comparing the use of various methods used in the preservation of platelets. The program has been extended for use by the Leukemia Task Force for large scale studies involving a number of therapy centers. A contract was initiated for development of an appropriate container bag for low temperature storage and this is now in production and being used by the NCI.

4. Central nervous system complications. Newly diagnosed patients with acute leukemia are being studied to determine whether there is any significance to the presence of small numbers of abnormal cells in the spinal fluid in the absence of symptoms of meningeal leukemia. Patients who have had symptomatic meningeal leukemia are being followed systematically after treatment to detect cellular changes that will foretell the onset of symptomatic meningeal leukemia. An improved technique for assessing the spinal fluid cells morphology have been developed.

Significance:

Continued attempts to control hemorrhage and infection in acute leukemia patients has resulted in a dramatic curtailment of hemorrhagic deaths, with only marginal improvement in the prevention and management of infectious complications during the last 5 years. Accordingly the

Significance: (continued)

approach to these two problems has taken divergent paths. Hemorrhage in leukemia is most frequently the result of thrombocytopenia and is well controlled in most instances by platelet transfusions. For this problem refinements in an established technique are in order, most notably the development of tests for donor-recipient platelet compatibility, and methodology for platelet preservation. Progress in both areas is being made in conjunction with studies of the Immunology Branch, NCI and the blood banks and laboratories of the American National Red Cross, the Children's Hospital of Philadelphia, and the Children's Cancer Research Foundation of Boston. Attempts to reduce infectious complications have followed two major courses which are experimental and unproved at present. First is the attempt to reduce exposure to infectious agents by reverse isolation and reduction of autochthonous microorganisms by antisepsis and antibiosis (see NCI #3416). The second is the replacement of depleted granulocytes through transfusion of normal or chronic leukemic leukocytes (also see NCI 3415). Both manuevers have been demonstrated to be practicable, although expensive and time consuming, and preliminary experience indicates that these techniques may prove of significant benefit for patients receiving radiation and chemical therapy. Proof of efficacy awaits carefully conducted comparative clinical trials. White cell transfusion studies would be greatly expedited if methods of preservation can be perfected. Studies to date have defined criteria of cell viability which are an important first stop in such studies.

The third major complication of acute leukemia is meningeal leukemic infiltration. Although the extent and characteristics of this complication have been frequently studied, most studies have relied on post-mortem examination. A precise and simple method of spinal fluid examination has been perfected during the last year and is currently being applied to studies of the natural history and cause of this complication in living patients.

Proposed Course:

1. Evaluation by controlled study of reverse isolation, gastrointestinal sterilization, granulocyte transfusions, hyperimmune plasma, and newly available antibiotics in the control of infection in acute leukemia.

2. Studies on the development of techniques for the cryopreservation of leukocytes and platelets.

3. Studies of central nervous system complications of leukemia and its treatment. This will include studies of the comparative effectiveness of cytosine arabinoside and methotrexate in the control of meningeal leukemia.

Part B Honors, Awards, and Publications

1. Pert, J.H., Zucker, M.B., Lundberg, A., Yankee, R., and Henderson, E.: Recent Advances in Preparation of Platelet Concentrates from ACD and CPD. Blood. Vox Sang. 13: 119-125, 1967.

2. Skeel, R. T., Yankee, R. A., Henderson, E. S.: Meningeal Leukemia: Two Simple Methods for the Rapid Detection of Malignant Cells in the Spinal Fluid. J. A. M. A. In Press

3. Albert, D.M., Wong, V.G., and Henderson, E.S. Ocular Complications of Vincristine Therapy. Arch. Ophthal. 78: 709-713, 1967.

Serial No. NCI-3407
1. Medicine Branch
2. Leukemia Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Part A

Project Title:
1) Studies on RNA synthesis in Human Lymphocytes
2) Studies on Virus Complementary RNA in Hybrid Virus Transformed Cells.
3) Studies on the Genetic Organization of Simian Virus 40.
4) Effect of Daunomycin on Nucleic Acid Synthesis of Primary Rat Heart Cultures.

Principal Investigator: Patrick H. Henry, M.D.

Other Investigators:
1) Umberto Torelli, Paul Neiman and Sherman Weissman
2) Myron Levin, Paul H. Black, Michael N. Oxman and Sherman Weissman
3) Myron Levin, Arthur Levine, Michael N. Oxman, Andrew Lewis, William Wiese, George Diamandopoulos, Wallace P. Rowe, John Enders
4) Joel Rubenstein

Cooperating Units:
1) Laboratory of Virus Disease, Lewis, Oxman, Weise, Rowe, NIAID
2) Department of Medicine, Infectious Disease Service, Massachusetts General Hospital, Dr. Black
3) Department of Medicine, Yale University School of Medicine, Dr. Weissman
4) Children's Hospital Medical Center, Boston, Mass. Drs.Enders and Diamandopoulos

Man Years (July 1, 1967 to June 30, 1968):

Total:	9
Professional:	8
Other:	1

Project Description:

Objectives:

1a) To study the *in vitro* synthesis and metabolism of RNA from both the unstimulated normal small lymphocyte and the PHA-stimulated lymphocyte.

Objectives: (continued)

1b) To compare by hybridization and competition experiments and acrylamide gel electrophoresis the RNA's synthesized by lymphocytes from CLL, ALL, AML, normal small lymphocytes and PHA lymphocytes.

2a) To study baby hamster kidney cells transformed by adenovirus-Simian Virus 40 hybrids for the presence of virus complementary RNA.

2b) To study the effect of various alterations in growth of virus transformed cells on the presence of virus messenger RNA.

2c) To study the genetic organization of Simian Virus 40.

Methods Employed:

Standard biochemical techniques have been employed for the measurement of DNA, RNA, and protein synthesis. Extraction procedures have been developed for the isolation of undegraded DNA and RNA. Techniques for hybridization studies of DNA and RNA have been employed. KB cells have been established in spinner culture. The use of agarose polyacrylamide in disc gel electrophoresis has been set up in the laboratory this year.

Major Findings:

1) The CLL lymphocyte has been shown to have species of DNA-like RNA that are not present in the normal small lymphocyte.

2) Various types of cells acutely infected and transformed by SV40 have been examined with hybridization competition studies to determine which portion of the virus genome codes for the various viral functions.

Proposed Course:

1) Further attempts will be made to characterize the RNA synthesized by normal and PHA lymphocytes with polyacrylamide gel electrophoresis, base analysis and hybridization competition studies.

2) The RNA metabolism in CLL and normal lymphocytes appears superficially rather similar. We will attempt to compare these two RNA's with more sensitive techniques of nucleic acid homology and gel electrophoresis. RNA from ALL and AML will also be compared with CLL and normal lymphocyte RNA.

3) Several studies are planned to determine if alterations in the amount of virus messenger RNA can be effected in the hybrid transformed cells.

Proposed Course: (continued)

4) Attempts will be made to fractionate adenovirus and SV40 DNA's in preparation for studying the various genetic functions of these oncogenic viruses.

Part B Honors, Awards, and Publications

1. A.E. Freeman, P.H. Black, E.H. Vanderpool, P.H. Henry, J.B. Austin, and R.J. Huebner: Transformation of Primary Rat Embryo Cells by Adenovirus Type 2. PNAS 58: September 1967.

2. U.L. Torelli, P.H. Henry, S.M. Weissman: Characteristics of the RNA Synthesized by the Normal Small Lymphocyte and the Changes Induced by Phytohemagglutinin Stimulation. JCI May 1968.

Serial No. NCI-3416
1. Medicine Branch
2. Leukemia Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: "Life Island" Isolator Study

Previous Serial Number: NCI-3416

Principal Investigator: John G. Hazen, M.D. (after April 15, 1968, Harvey D. Preisler, M.D.)

Other Investigators: Seymour Perry, Corwin D. Strong, Wilmer Dockstader, Lloyd Herman, Florence Seidler, Janet Rueter, Richard Asofsky, Leonard Laster, Louis Bourgeois, Helen Eliot, and Andrew Vargosko (succeeding Dr. Vargosko after January 27, 1968, is Dr. Robert Chambers).

Cooperating Units: Acute Leukemia Service and Solid Tumor Service, M.B., NCI. Environmental Sanitation Control Department, Pharmacy Department, Patient Dietetic Service, Physical Therapy, Occupational Therapy Service, Central Sterile Supply Service, Nursing Service, and Infections Laboratory Services, Department of Clinical Pathology, Clinical Center, also Environmental Services Branch, DRS.

Man Years

Total: 5
Professional: 3
Other: 2

Project Description:

Objectives

To evaluate the effect of a germ-free environment upon patients undergoing intensive chemotherapy. One alteration during the past year has been the decision to randomize untreated cases of acute myelogenous leukemia between 15 and 60 years of age to (1) care within the patient isolator enclosure vs. (2) care in the open hospital rooms with the addition of sterile food and non-absorbable antibiotics. In addition, a third group will be made up of those patients receiving optimal antileukemia therapy (as will the first two groups) but without the addition of the patient isolator, sterile food or non-absorbable antibiotics. Also eligible are patients in the blastic phase of chronic granulocytic leukemia.

Methods Employed

A plastic isolator system incorporating an air filtration system is used to isolate the patient from his surrounding environment. Prior to placement into the isolator and during the period of isolation, the patient receives antibacterial scrubs and oral non-absorbable antibiotics designed to reduce his normal gastrointestinal flora.

Major Findings

1. From July 1, 1967, to the present (April 5, 1968), five patients have been placed within the isolator. Two of these patients had aplastic anemia, one metastatic choriocarcinoma, one acute lymphocytic leukemia, and one acute myelogenous leukemia (the first patient on the new randomization study). Striking therapeutic benefit has occurred in two patients, but we cannot solely attribute this to the "Life Island" enclosure. The possibility remains that these patients may have done as well outside the enclosure. One of these patients, Mrs. S.E., with metastatic choriocarcinoma has now been free of any evidence of tumor for six months following removal from the isolator. She spent 59 days within the isolator. The other patient, Mr. K.C., with the diagnosis of aplastic anemia, has returned to being a full time student and no longer has a transfusion requirement of either red cells or platelets. He remained within the isolator a total of 203 days and had no systemic infection. The other patient with aplastic anemia expired unexpectedly from internal hemorrhage after 35 days within the "Island." The patient with acute lymphocytic leukemia was unimproved after a 31 day stay within the "Island." The patient with acute myelogenous leukemia left the "Life Island" after 90 days with a partial remission.

2. During the year, the following infectious episodes occurred: a) A Staph epidermidis paronychia of the great toe in the patient with aplastic anemia who improved and requires no further blood product transfusion. The paronychia resolved in thirteen days. b) The patient with acute lymphocytic leukemia developed Candida septicemia within the "Island" under circumstances difficult to control. After receiving total body irradiation and high doses of antimetabolites, the patient had marked nausea and vomiting. He was unable to ingest the oral non-absorbable antibiotics, which included Mycostatin. Six days after going off the oral antibiotic regimen, the patient developed Candida septicemia while still within the enclosure.

Proposed Course

A controlled study of the effect of the "Life Island" program is underway. Patients receiving the full protocol will be compared to patients receiving a) the sterile food and oral antibiotic measures without isolation and air filtration and b) patients receiving standard ward care, all groups receiving comparable chemotherapeutic regimens. Patients in this study will

will be restricted to adults with acute myelocytic leukemia. A collaborative effort is anticipated with participation by institutions having comparable reverse isolation facilities, e.g., M.D. Anderson and State University of New York (Coney Island Division).

Publications

1. Hazen, J.G., Levitan, A.A., and Bourgeois, L.D. Patient Isolator Systems in Cancer Chemotherapy: The Use of High Dose "Non-absorbable" Antibiotics in the Reduction of Bowel Flora. Am. J. Gastroenterology (In Press).

2. Levitan, A.A., Perry, S. The Use of an Isolator System in Cancer Chemotherapy. Am. J. Med. 44: 234-242, February 1968.

3. Levitan, A.A., Seidler, F.M., and Rueter, J.L. The Role of Supportive Services in the Operation of an Isolator System. J.A.M.A. 203, no. 12: 1009-1014, March 18, 1968.

4. Levitan, A.A. In Vitro Antibacterial Activity of Kasugamycin. Applied Microbiology 15, no.4: 750-753, July 1967.

Serial No. NCI-3419
1. Medicine Branch
2. Leukemia Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Part A

Project Title: Immunosuppression and Transplantation

Principal Investigator: Brigid Leventhal and Ronald Yankee

Other Investigators: Edward S. Henderson, Frank C. Grumet, C. Dean Buckner, and Robert G. Graw, Jr.

Cooperating Units: Laboratory of Human Genetics, NIDR
Laboratory of Bacterial Products, DBS
Immunology Branch, NCI

Man Years:

Total: 3
Professional: 2
Other: 1

Project Description:

Objectives:

1. To study functional properties of leukocytes *in vitro*.
2. To assess the use of marrow stem cell grafting as an adjunctive measure in leukemia therapy.
3. To investigate the existence of immunity against tumor systems by delayed hypersensitivity and *in vitro* lymphocyte transformation.
4. To assess the optimal methods of obtaining isogeneic and allogeneic grafts in human leukemia patients.
5. To evaluate the effects of immunosuppressive drugs on host immunity and graft acceptance and rejection phenomena.

Major Findings

We have attempted to define the ideal conditions for *in vitro* lymphocyte response and have studied the effect of cell density on lymphocyte cultures. Higher cell density is necessary for response to antigen than for response to phytohemagglutinin. We found also in the course of this study that removal of macrophages from in vitro cultures does diminish lymphocyte responsiveness to antigen but that this response can be restored by readdition of phagocytic cells or partially restored by increasing the density of purified lymphocytes.

Other evidence that a proportion of lymphocytes may have a macrophage-like function was gathered in our studies with cultures stimulated with PHA and M. pneumoniae where autoerythrophagocytosis by a small percentage of lymphoblasts was seen. In the course of our studies on M. pneumoniae, we became aware of reports in the literature that M. hominis and M. orale inhibit the effect of PHA in culture. We were not seeing inhibition with M. pneumoniae, Mycoplasma can be divided into two classes on the basis of metabolic requirements: those which require arginine for energy, such as M. hominis and M. orale, and those which require dextrose (fermenters) such as M. pneumoniae. Investigation of several mycoplasma species in our laboratory has shown that the arginine using strains inhibit PHA stimulation and that this effect is reversed by addition of arginine while the dextrose users do not inhibit.

An active white cell typing program has been started in conjunction with the Immunology Branch. One 12 year old identical twin with acute lymphocytic leukemia was treated with intensive chemotherapy followed by radiation. Bone marrow cells from his mother, who resembled him closely by leukocyte antigen studies, were then infused. It was hoped that a nonrelated graft might be established which would have some activity against his own leukemia cells, but that if graft versus host reaction (GVH) became a problem, the maternal graft could be ablated and an identical twin graft substituted. The patient expired several days after the maternal marrow had been infused. It was unclear whether his death was due to GVH or cumulative drug toxicity.

Significance:

We feel that we have gone further in defining lymphocytes with more than one type of function, particularly in identifying some which appear to have a phagocytic capacity.

The work with the arginine using mycoplasma was entered into merely to explain an apparent difference between our data and that of others, however, the cultures grown with the arginine using cells have been interesting in that the arginine depleted cells do not grow but are not killed, implying that this amino acid may be essential for cell division but not survival.

Proposed Work:

We will continue our work on in vitro lymphocyte function. We hope to utilize an agar system where individual lymphocytes can be isolated and studied during response to PHA and other stimuli. We are also actively investigating the best techniques for lymphocyte transformation in several subhuman species (mice and baboons) hoping that we may be able to use this as a technique for identifying the antigens which cause delayed hypersensitivity type reactions in some carcinogen induced tumors and extend this later to a study of skin tests and the reaction of human leukocytes to membrane antigen prepared from leukemic leukocytes in cooperation with the Immunology Branch.

Any opportunity for isogeneic marrow grafting will be vigorously pursued. The transfusions of chronic myelogenous leukemia leukocytes will be continued and any grafts that are established will be investigated histochemically and for their participation in the inflammatory response, to determine the effect of the host upon the graft as well as the effect of the graft upon the host.

The problems of leukocyte procurement and preservation are also under investigation in our unit and when a reliable method of bone marrow preservation is established a program of elective grafting in patients with leukemia will be initiated.

Part B Honors, Awards and Publications

1. Leventhal, Brigid G., and Oppenheim, Joost J. Effect of Cell Density on the Rate and Degree of Response of Leukocyte and Purified Lymphocyte Cultures. Proceedings of the Third Annual Leukocyte Culture Conference, W. O. Rieke (ed.) Appleton Century Crofts, New York (in press).

2. Leventhal, Brigid G., Smith, Charles B., Carbone, Paul P., and Hersh, Evan M. Lymphocyte Transformation in Response to *M. pneumoniae*. (ibid)

3. Handmaker, Stanley D., Leventhal, Brigid G., and Cooper, Herbert L. The Kinetics of PHA Stimulation of Human Lymphocytes. (ibid)

4. Oppenheim, Joost J., Leventhal, Brigid G., and Hersh, Evan M. The Transformation of Column Purified Lymphocytes with Nonspecific and Specific Antigenic Stimuli. J. Immunol. (in press).

Serial No. NCI-3403
1. Medicine Branch
2. Solid Tumor Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Clinical Trials of Chemotherapeutic Agents in Patients with Solid Tumors

Previous Serial Number: Same

Principal Investigators: Paul P. Carbone, Vincent T. DeVita, George P. Canellos

Other Investigators: Charles L. Vogel, Martin H. Cohen, John L. Ziegler, Charles M. Haskell

Cooperating Units: NCI-VA Cancer Research Unit, Washington Veterans Administration Hospital, Washington, D.C.
U.S. Public Health Service Hospital, Baltimore, Maryland

Man Years:

Total:	6
Professional:	1
Other:	0

Project Description:

Objectives:

The objectives of these studies are to improve the treatment of human cancer with particular emphasis on problems related to lymphosarcoma, Hodgkin's disease and selected solid tumors. The selection of agents for clinical trials is derived mainly from various animal tumor screens, particularly from information derived from tumor cell kinetic oriented screens. The clinical studies are designed not only to give basic information regarding antitumor effect but also improve the experimental design of clinical studies.

Methods Employed:

All patients on the Solid Tumor Service are potential candidates for study.

Major Findings:

A. Combination Chemotherapy of Hodgkin's Disease. Continued evaluation of a combination of four drugs, vincristine, procarbazine, nitrogen mustard and prednisone is in progress. The four agents are given together in cycles lasting ten days followed by a two week rest period. A full course of therapy is six cycles and takes six months. Following the six cycles no further therapy is given. Careful attention is paid to the completeness of remission, which is re-evaluated by repeating appropriate studies at intervals during and after therapy.

At the present time, 65 patients have been or are still being treated. As of March, 1968, the complete remission rate with the combination was 83 per cent; the duration of unmaintained complete remissions (calculated from the first 30 patients) was 18+ months.

Toxicity is tolerable enough so that patients receive most of their therapy as out patients; neurologic toxicity from vincristine has been reversible with time. In July, 1967, the protocol was modified to randomize patients into one of two groups: 1. no maintenance therapy, and 2. maintenance therapy.

Particular attention is now being devoted to the duration of unmaintained remissions in the initial group. To date, they have been considerably longer than any single drug therapy thus far examined.

B. Clinical Trials - Lymphosarcoma - Reticulum Cell Sarcoma. Combination chemotherapy of lymphosarcoma and reticulum cell sarcoma is under study. Remission induction is attempted with five consecutive days of therapy separated by 16 to 20 days of no therapy. Each cycle consists of the following three drugs given intravenously:

vincristine	0.3 mg/m^2 daily x 5
cyclophosphamide	400 mg/m^2 daily x 5
prednisolone	100 mg/m^2 daily x 5

Patients achieving complete remission after four cycles receive two additional cycles at the same time intervals for consolidation of remission. All complete remissions will then be randomized to maintenance therapy every two months, with a single cycle or no maintenance therapy.

To date 11 patients have been entered on study. Two have completed remission induction therapy. One of these has achieved complete remission although his therapy was temporarily interrupted because of the discovery and surgical removal of a carcinoma of the colon. The other patient has one palpable lymph node remaining after therapy which is to be biopsied shortly. Eight of the remaining patients are still receiving remission induction cycles and all have had dramatic tumor shrinkage during therapy. One patient died of gram negative septicemia on the third day of the first cycle. At post mortem all tumor tissue was necrotic. Most patients have suffered alopecia and some nausea and vomiting during therapy. Three patients have developed leukopenia to <1500 WBC/cc all after the first cycle of therapy. Further cycles in two of these patients were well tolerated with cyclophosphamide dosage decreased to 3/4 the initial dose.

C. Burkitt's Tumor Therapy. Ten patients have been entered on the Burkitt Tumor Treatment Protocol. These patients receive cyclophosphamide 40 mg/kg every three to four weeks for six or more courses. If in remission at the completion of this treatment they are given no maintenance therapy. All but one patient has completed this therapy. All received six or fewer courses of cytoxan except one patient who received ten courses of therapy.

Five patients are now in unmaintained remission with a mean survival from the time of diagnosis of 14.6 months to the present time. One patient has relapsed after being in unmaintained remission for six months. The remaining patients have expired. The ongoing mean survival of 14.6 months in this group of patients contrasts to our experience in the first nine patients with Burkitt tumor treated at the NCI with a variety of chemotherapeutic agents and x-ray where the mean survival was 2.5 months.

D. Effects of Cytoxan and Lysosomes Labelizers on L1210 Cells. Release of hydrolytic enzymes contained in lysosomes may lead to cell death. In this regard simultaneous release of lysospmal enzymes from large quantities of cells may also lead to death of surrounding cells. Studies with L1210 leukemia cells were undertaken to determine first if a chemotherapeutic agent would increase the intracellular content of liposomal enzymes and second whether a liposomal labilizer would cause the rapid release of these enzymes extracellularly. Acid phosphatase levels in L1210 cells were measured before and after cyclophosphamide. Following parenteral cyclophosphamide administration intracellular acid phosphatase was found to increase at least twofold. Subsequent *in vitro* experiments using these cells and vitamin A as a lysosomal labilizer indicated that vitamin A did indeed cause a rapid release of acid phosphatase into the medium when compared to controls. These studies are still in a preliminary phase. It is hoped to extend them to solid tumors which theoretically should be more sensitive to local release of large quantities of liposomal enzymes than cells in ascitic fluid.

E. Clinical Trials NSC 82196. (Imidazole carboxamide bis chlorethyl triazeno. Phase I study). The antitumor agent, NSC 82196, has been entered into Phase I clinical trials. The following dosage schedules are to be evaluated: (a) weekly 400 mg/m^2, 800 mg/m^2, 1200 mg/m^2, and (b) five day courses every three weeks at 100 mg/m^2/day, 200 mg/m^2/day and 300 mg/m^2/day. To date four patients have received 400 mg/m^2/week; three, 800 mg/m^2/week; three, 100 mg/m^2/day; and three, 200 mg/m^2/day.

There have been no therapeutic responses to date. No liver toxicity was noted in any patient. Mild to moderate nausea and/or vomiting was experienced by all three patients at 800 mg/m^2/week. Mild leukopenia was noted in one patient (range 3,000-4,000/mm^3) while on 800 mg/m^2/week. If toxicity at 800 mg/m^2/week and 200 mg/m^2/day x 5 is not severe and if no delayed toxicity is noted, the present plan is to begin new patients on the next highest dose schedule, either 1200 mg/m^2/week or 300 mg/m^2/ week x 5.

F. Clinical Trials. Studies with oral 1,3 bis (2-chloroethyl) t-nitrosourea (BCNU) and the combination of cytosine arabinoside (CA) with parenteral BCNU. Previous studies indicated that single doses or short courses (three days) of BCNU, while producing significant toxicity, were safe for clinical use. The drug is absorbed orally but studies on clinical toxicology by the oral route have not been available. Recently, good evidence of drug synergism between BCNU and CA in the experimental tumor system leukemia L1210 has suggested that this combination might have some clinical usefulness.

Studies have been in progress, therefore, to evaluate the use of oral BCNU alone and BCNU and CA in combination.

1. Oral BCNU. Five patients were given oral BCNU from 20 to 60 mg/m^2 every fourth day. Sixty milligrams per square meter produced toxicity after eight doses that was reversible. No clinical responses were seen in these patients. (Three melanomas and two carcinomas of the lung). The appearance of toxicity with the oral use of the drug coupled with prior pharmacologic information indicates that the active form of the drug is absorbed intact. No liver toxicity was noted by the oral route. These studies served as the basis for dose selection in Part 2 below.

2. BCNU and CA. Eighteen patients have been given this combination of drugs. Studies were initiated at doses of 40 mg/m^2 of BCNU and 100 mg/m^2 of CA and as experience accumulated the dose was increased.

   It has been found that BCNU and CA can be given in doses of 40 mg/m^2 and 225 mg/m^2 respectively, every fourth day for six to 12 doses with reversible marrow toxicity. Both drugs are given parenterally. Liver toxicity has not been a significant problem. Ten of the 12 patients were in the blast phase of chronic myelogenous leukemia; while almost all these patients had hematopoietic suppression, only two patients had useful clinical remissions; one complete remission of acute blast crisis and one reversion to the chronic phase of the disease. The six patients with solid tumors showed no response, other than toxicity, to the drug.

3. BCNU in Hodgkin's Disease. Ten patients with advanced Hodgkin's disease, resistant to all other known effective drugs (including alkylating agents) were given BCNU, 200-250 mg/m^2 intravenously. Six of the ten patients had good to excellent responses while four

had no response or progression of their disease. Four of the six responders have remained in partial remission with repeated doses of BCNU for periods up to two years. It appears that BCNU is not clinically cross resistant to alkylating agents and is a useful drug in the management of patients with Hodgkin's disease.

G. L-Asparaginase

1. Treatment of neoplastic disease

A protocol study of the effect of L-asparaginase treatment on solid tumors is underway. Four patients have received the enzyme. Two patients with melanoma; one male choriocarcinoma and one osteosarcoma. Significant objective and subjective improvement occurred in one of the two melanoma patients. The other melanoma patient had slight objective regression. The remainder are still on therapy.

2. Short term incubation studies of tissues from patients with neoplasia for the study of asparagine dependence.

Cell preparations from five patients with granulocytic leukemia, two with lung cancer, two melanoma, one osteosarcoma and one mycosis fungoides were obtained. Asparagine dependence was noted in one melanoma who was also sensitive to the drug asparaginase on clinical study. Variable sensitivity was noted in leukemia. The others were negative.

3. L-asparaginase levels in patients on therapy.

Assay of L-asparaginase in seven of the patients on study is underway to determine effects of dose on blood levels.

4. *In vitro* study of L-asparaginase on leukemia leukocyte and erythrocytes.

Preliminary data indicate lack of transport into leukemic granulocytes and red cells. No alteration of red cell membrane as determined by osmotic fragility studies was noted. It appears that the effect of the enzyme is to deplete extracellular asparagine with secondary lethal effect on those cells unable to synthetase in malignant tissue is under study.

H. Purine Nucleosides and 5-Fluorouracil Cytotoxicity. Demonstration *in vitro* that purine nucleoside (inosine) will enhance uracil incorporation into RNA prompted an animal study to test the effect of inosine on 5-FU toxicity. Animals (mice) with P388 leukemia demonstrated enhanced toxicity of 5-FU after pretreatment with inosine. Animals died, free of tumor, of 5-FU toxicity. Further studies are planned at lower doses. The possibilities that inosine infusions into patients directly or intracavitary injections may enhance 5-FU effects on tumors. The mechanism of increased 5-FU is presumed to be secondary to increased activity of **uridine phosphorylase.**

Honors and Awards: None

Publications:

1. DeVita, V.T., Carbone, P.P., Owens, A.H., Gold, G.L., Krant, M.J. and Edmonson, J.: Clinical trials with 1,3 bis (2 chloroethyl)-1-nitrosourea. Cancer Research 25: 1876-1881, 1965.

2. DeVita, V.T., Denham, C., Davidson, J.D. and Oliverio, V.T.: The physiologic disposition of the carcinostatic 1,3 bis (2 chloroethyl)-1-nitrosourea (BCNU) in man and animals. Clinical Pharmacology and Therapeutics 8:566-577, 1967.

3. Roberts, W.C., Clancy, D.L. and DeVita, V.T.: Heart and malignant lymphoma (Hodgkin's disease, lymphosarcoma, reticulum cell sarcoma, and mycosis fungoides). A study of 196 autopsied patients. American Journal of Cardiology (In Press).

4. Nordlund, J.L., DeVita, V.T. and Carbone, P.P.: Severe vinblastine toxicity during pregnancy with delivery of a normal infant. Annals of Internal Medicine (In Press).

5. Carbone, P.P.: Current approaches to the management of patients with malignant lymphoma. Maryland State Medical Journal, May 1967.

6. DeVita, V.T. and Carbone, P.P.: Lymphosarcoma. Con, H.F. (Ed.): Current Therapy. Philadelphia, Pennsylvania, W.B. Saunders Company, 1968, pp. 265-268.

7. Burke, P.J., Serpick, A., Carbone, P.P. and Tarr, N.: Cytosine arabinoside (NSC-63878): A clinical evaluation of dose and schedule of administration. Cancer Research 28: 274-279, 1968.

8. Carbone, P.P., Bell, W.R., Godwin, H. and O'Conor, G.T.: New approaches to the treatment of Burkitt-type tumor. Burchenal and Burkitt (Ed.): Treatment of Burkitt's Tumour. UICC Monograph Series 8: Springer-Verlag, Heidelberg, Germany, 1967, pp. 184-191

Serial No. NCI-3404
1. Medicine Branch
2. Solid Tumor Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Infection, Immunity and Cytogenetic Studies in Malignant Disease

Previous Serial Number: Same

Principal Investigators: Paul P. Carbone, Vincent T. DeVita, Jacqueline Whang, George P. Canellos

Other Investigators: Martin H. Cohen, Charles L. Vogel, Robert Eagen, Bruce Chabner, Edgar W. Hull

Cooperating Units: Pathologic Anatomy Branch, NCI
Dermatology Branch, NCI

Man Years:

Total:	6
Professional:	5
Other:	1

Project Description:

Objectives:

1. To study the effect of malignant disease and chemotherapy on host defense mechanisms.

2. Cytogenetic studies in patients with chronic myelogenous leukemia.

3. To develop laboratory models for following solid tumor growth kinetics.

Methods Employed:

Case material: All patients on the Solid Tumor Service are potential candidates for these studies.

Laboratory methods: Standard bacteriological, serological and biochemical methods were employed as well as new specific tests devised.

Major Findings:

A. Immunology of Hodgkin's Disease. A study of the immunologic aspects of 50 patients with Hodgkin's disease was previously published. This study has been extended to a total of 100 patients. The results are not significantly different. Seventy-five per cent of the patients of all stages respond to dinitrochlorobenzene (DNCB) sensitization. Stage I patients were normal; Stage IV patients were the least reactive. Sixty-three per cent of all the patients reacted to multiple skin tests. Lymphocyte transformation is more variable than tests of delayed hypersensitivity. Studies on the lymphocyte in Hodgkin's disease have been extended to studies of their ability to incorporate ^{3}H thymidine and ^{3}H uridine with and without phytohemagglutinin, sequentially over a period of five days.

B. Immunologic Studies in American Patients with Burkitt's Tumor. Evidence indicating active host resistance in African patients with Burkitt's tumor prompted a study of immunologic reactivity in ten American patients with tumors histologically and cytochemically identical with Burkitt's tumor in Africa. Delayed hypersensitivity was determined by dinitrochlorobenzene sensitization, intradermal testing to a variety of antigens including PPD, candida, trichophyton, mumps, and histoplasmin, and lymphocyte transformation with phytohemagglutinin. Normal responses to at least one of these tests were seen in all ten patients. In studying humoral aspects of immunity serum immunoglobulin levels were measured and patients received primary immunization with Vi antigen. Normal serum levels of IgG were noted in five patients with decreased levels in five patients. Two of the latter patients had, however, a normal use in antibody titer following immunization with Vi antigen. The serum levels of IgG may correlate with response to therapy as seen by the fact that the three patients with the lowest levels of IgG all had poor responses to therapy. These three patients also had decreased concentrations of at least one of the other two major immunoglobulin classes IgA and IgM.

Reticuloendothelial system phagocytosis was measured with iodinated microaggregated human serum albumin. Normal clearance rates were noted in four patients with increased rates in three and decreased rates in two patients. No correlation could be made between reticuloendothelial phagocytosis and response to therapy.

C. Reticuloendothelial System Function. A correlation appears to exist between host resistance to malignant tumors and the functional status of the reticuloendothelial system. At present we are studying the phagocytic and metabolic function of the reticuloendothelial system in patients with a variety of malignant diseases using iodinated microaggregated human serum albumin as the test colloid. In addition the effects of a variety of cancer chemotherapeutic agents on the reticuloendothelial system are being studied. Results thus far indicate that in patients with Hodgkin's disease and other reticuloendothelial malignancies both the phagocytic and catabolic functions of the reticuloendothelial system are enhanced. In contrast preliminary studies in patients with tumors such as bronchogenic carcinoma indicate that both phagocytosis and catabolism by reticuloendothelial cells are depressed. Preliminary study of the effect of chemotherapy on reticuloendothelial function indicates that while phagocytic rates remain essentially unchanged the catabolic functions of reticuloendothelial cells are depressed. Future studies will attempt to quantitate the degree of depression of catabolism and also its duration.

D. Infection and Immunity Section. Clinical records of 2,887 consecutive, unselected patients in the autopsy file of the NIH were reviewed. Seven cases of fatal Pneumocystis carinii pneumonia were found. Six of these had underlying chronic diseases and had been on long-term prednisone therapy. Five had malignant disease of the reticuloendothelial system.

The diagnosis of pneumocystosis was suspected antemortem in two patients, and treatment was instituted with pentamidine isothionate. In both cases the duration of treatment was short so that full evaluation of the treatment efficacy of pentamidine could not be made.

Predisposing factors, presenting clinical and roentogenographic features of the disease, and attempts at diagnosis and therapy are discussed. The need for vigorous attempts at early definitive diagnosis was stressed. At present, lung biopsy appears to be the most reliable diagnostic procedure. The effectiveness of pentamidine isothionate in treating clinical Pneumocystis carinii pneumonia can be evaluated only by early institution and adequate trial of therapy in patients with an established diagnosis.

Serologic survey for toxoplasmosis in patients with malignant disease (done in conjunction with Lunde, M.N. and Melton, M. of NIAID).

Toxoplasma HA tests were performed, postmortem, on 40 patients with malignant diseases of the reticuloendothelial system and on 240 additional patients antemortem. Variables analyzed included morphological diagnosis, state of residence, gamma globulin levels, and relationship of the date of serologic determination to administration of antineoplastic chemotherapy and/or blood products.

There were no significant differences in the percentage HA positivity between our antemortem series and similar studies done on otherwise normal United States populations where an increase in per cent positivity with age was observed. Patients with chronic myelogenous leukemia tended to have higher titers than patients in other disease categories. Seroconversion from negative to positive during therapy for malignant disease was observed. We have shown that some of these cases of seroconversion were caused by transfusion of blood products.

E. As part of a continuing review of infectious complications of cancer chemotherapy, the records of all autopsies at the NIH have been reviewed to ascertain the incidence and distribution of superinfection with aspergillus fumigatus.

Ninety-five patients were found with proven infection; all but ten patients had a hematological malignancy. Particular attention was paid to pulmonary involvement because of the frequent appearance of a typical lobar pneumonia from aspergillosis in our patients. Only seven of the 95 patients did not have pulmonary involvement. Commonly, aspergillosis of the lung presents as a patchy pneumonitis or with complete lobar consolidation.

This is contrasted to the types of infections usually seen in patients with non-malignant disease, described as an allergic pneumonitis or invasion by the organism of open negative old tuberculosis cavities.

F. Immunosuppression. At present no systemic evaluation of the immunosuppressive capabilities of potentially useful antineoplastic compounds has been undertaken by the CCNSC. We are evaluating Experimental Allergic Encephalomyelitis (EAE), a readily reproducable disease of delayed hypersensitivity which might well prove useful as a screening procedure for immunosuppressive potential of chemotherapeutic agents. On day zero, Lewis rats are innoculated intradermally in a single hind foot pad with a purified encephalitogenic protein (isolated from guinea pig spinal cord) emulsified in Freund's adjuvant. Pertussis vaccine is given in the dorsum of the same foot. Drugs are given in approximately equitoxic doses in the following schedules:

A. Daily for 5 days prior to day 0
B. For 5 days starting on day 0
C. For 15 days starting on day 3 after innoculation
D. For 5 days starting on day 8 (just prior to clinical manifestations of disease

To date three agents have been tested (cyclophosphamide, procarbazine, and NSC 82196). Nineteen of nineteen untreated control animals developed unequivocal hind limb paralysis between days 9 and 12 after innoculation. Twenty-five additional animals treated with NSC 82196 also developed EAE by day 12. In contrast 6/6 animals treated with schedule E of cyclophosphamide survived, free of disease until day 20 and 4/6 animals treated with procarbazine in schedule C survived free of disease for greater than 35 days. Other dosage schedules of cyclophosphamide and procarbazine provided definite but lesser degrees of protection. Histologic sections of central nervous system tissue are pending and should help clarify whether some late deaths were due to EAE or drug toxicity. To date the results are very encouraging and point to the possible usefulness of this model as a screen for immunosuppressive potential in antineoplastic agents.

G. α-Fetoprotein in Monkeys with Hepatoma. An αglobulin, α-fetoprotein (αFP), is found in sera of fetal and newborn mammals and in humans and rodents with hepatoma, but not in normal adult sera. In 82 monkeys treated with several hepatic carcinogens, we tested for αFP in the serum, studied the time relation of αFP appearance to liver function test (LFT) abnormalities and physical signs of tumor, and attempted to correlate αFP production with histopathology. αFP assays, using immunodiffusion with specific anti-αFP rabbit serum, LFT's and abdominal palpation were done monthly. Open liver biopsies were performed on monkeys with nodular hepatomegaly and/or positive αFP assays. Twenty-two of 57 monkeys treated with N-nitro-sodiethylamine (DENA) and one of 35 monkeys receiving other carcinogens have developed biopsy-proven hepatomas. αFP was found in 17 of 23 monkeys (74%) with hepatomas, including 35% with drug-induced liver disease. There was no apparent correlation between the degree of cellular differentiation of tumor and the appearance of serum αFP. However, in three of the six αFP-negative monkeys with hepatoma, a heavy lymphocytic infiltration was noted in the tumor. In some cases, αFP was detected before tumor was grossly palpable. Thus, αFP is also a specific marker for hepatomas in monkeys.

Awards and Honors: None

Publications:

1. Fraumeni, J.F., Vogel, C.L. and Easton, J.M.: Sarcomas and multiple polyposis in a kindred. Archives of Internal Medicine 121: 57-61, January 1968.

2. Strober, W., Wochner, R.D., Carbone, P.P. and Waldmann, T.A.: Intestinal lymphangiectasia: A protein-losing enteropathy with hypogammaglobulinemia lymphocytopenia and impaired homograft rejection. J. Clin. Invest. 46: 1643-1656, October 1967.

3. Ziegler, J. and Carbone, P.P.: Immunologic studies in American Patients with Burkitt-like lymphoma. Cancer Research 27: 2527-2531, 1967.

4. Tanaka, Y., Bell, W.R., Brindley, D.: Pseudoviral inclusion bodies in acute leukemia. A report of two cases. JNCI 38: 629-638, May 1967.

5. Vogel, C.L., Cohen, M.H., Powell, R.D. and DeVita, V.T.: Pneumocystis carinii pneumonia. Annals of Internal Medicine 68: 97-108, 1968.

6. Carbone, P.P.: Lysomes, Cortisone, and Aspergillosis. Annals of Internal Medicine 68: 708-709, March 1968.

7. Carbone, P.P. and Spurr, C.: Management of patients with malignant lymphoma: A comparative study with cyclophosphamide and vinca alkaloids. Cancer Research 28: May 1968.

8. Brown, R.S., Haynes, H.A., Foley, H.T., Godwin, H.A., Berard, C.W. and Carbone, P.P.: Hodgkin's disease: Immunologic clinical and histologic features of 50 untreated patients. Annals of Internal Medicine 67: 291, 1967.

Serial No. NCI-3405
1. Medicine Branch
2. Solid Tumor Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Multiple Myeloma and Chronic Leukemia; Clinical and Experimental Studies

Previous Serial Number: Same

Principal Investigators: Paul P. Carbone, George P. Canellos, Jacqueline Whang

Other Investigators: H. Thomas Foley, Paul E. Neiman, John Bennett

Cooperating Units: Clinical Pathology Department, NIH

Man Years:

Total:	5
Professional:	4
Other:	1

Project Description:

Objectives:

To investigate the relationship of the Philadelphia chromosome to the natural history and treatment of chronic myelogenous leukemia.

To improve methods of treatment for patients with CML, the chronic and acute phases.

Methods Employed:

A. Serial cytogenetic studies were performed on 179 chronic myelocytic leukemia patients (CML) at the Clinical Center of the National Institutes of Health. Patients were divided into six groups according to cytogenetic findings: Ph' negative (18 patients), Ph' negative with aneuploidy (3 patients), Ph' positive (116 patients), Ph' positive and negative (10 patients), Ph' positive with aneuploidy (24 patients), and Ph' positive which turned aneuploid (8 patients). Patients who were Ph' negative and negative with aneuploidy (11.8% of total) had the shortest median survival, 12-15 months. In contrast, the median survival of patients in the Ph' positive groups were 31-40 months. The development of aneuploidy in Ph' positive patients presaged the terminal stage of the disease.

B. The Philadelphia (Ph') chromosome in chronic granulocytic leukemia (CGL) is the only specific cytogenetic defect in human neoplastic disease. Attempts to demonstrate a definite biochemical lesion associated with this abnormality have not as yet been successful. Out of a group of 180 consecutive patients admitted to the National Cancer Institute with clinical and hematologic features of CGL, 20 (12%) Ph' negative adults were studied. The mean age, sex distribution and presenting symptomatology did not differ significantly from the larger Ph' positive group. One patient had leukemia cutis. The majority (15/20) had splenomegaly of some degree. There was a higher incidence of etiologic factors possibly related to leukemogenesis. Marrow morphology did not distinguish between the two groups. Of interest was the presence of myelofibrosis in two patients, one of whom evolved to polycythemia vera. The median peripheral white blood and platelet counts were 40,000/mm^3 and 2.6,500/mm^3 respectively and were significantly lower than the Ph' positive CGL. Leukocyte alkaline phosphatase levels were below normal in 16/20 cases. Serum B_{12} was elevated in 3/8 patients studied. The highest B_{12} levels appeared in the patients with highest white blood counts.

Lack of the Ph' chromosome seemed to indicate a graver prognosis with refractoriness to chemotherapy and early transformation to the acute blast form. Of the 15 who received chemotherapy only three demonstrated a good hematologic response, and these represent the longest survivors of the group. The median survival was 34 months as compared with 45 months in the Ph' positive group.

C. DBM-Myleran Study. In January, 1967, a new prospective study was begun to test the comparative efficacy of busulfan and a new agent, dibromomannitol (DBM), in the treatment of chronic myelogenous leukemia. Previous Phase II clinical studies had indicated that DBM was an effective and useful agent for the treatment of CML. The new study was designed carefully to measure its usefulness against the standard form of treatment for this disease and thereby establish its place in the regular management of the disease. Ancillary questions related to chronic toxicity resultant from the long term use of such agents in a relatively chronic disease such as CML are also being approached with this study. Patients with CML who have had no previous treatment are being randomized into two groups, one to receive DBM and one busulfan. They are compared in regard to the ability of the respective drugs to produce a remission, as defined by a return to normal peripheral blood and bone marrow morphology, and by their ability to maintain such remission. The correlation of leukocyte alkaline phosphatase rise and serum B_{12} level fall with such responses is also being made. Toxicity is being monitored by following hematologic parameters, liver and renal function, bladder sputum and cervical cytology, cutaneous pigmentation, and the occurrence of cytogenetic changes in both groups. Patients developing resistance or unacceptable toxicity to either drug will be switched to the alternate mode of therapy to determine the extent of cross resistance or shared toxicity between the agents. Ultimately the comparative effects of the drugs on morbidity and longevity will be assessed.

To date fifteen patients have been entered on the study, eight on DMB and seven on myleran. The data are still too preliminary to draw any conclusions. Six of the patients on DBM and two of the patients on busulfan have achieved ample remission and are on maintenance therapy. Three patients have entered blast crisis and gone off study and the rest have had partial responses which are being maintained. There have been no therapy related deaths and one admission for therapy (busulfan) related toxicity.

Awards and Honors: None

Publications:

1. Whang, J., Canellos, G.P., Carbone, P.P. and Tjio, J.H.: Clinical implications of cytogenetic varients in chronic myelogenous leukemia. *Blood* (In Press).

2. Lazarus, G.S., Brown, R.S., Daniels, J.R. and Fullmer, H.M.: Human granulocyte collagenase. *Science* 159: 1483-1485, March 1968.

3. Fishbein, W.: Excretion and hematologic effects of single intravenous hydroxyurea infusions in patients with chronic myeloid leukemia. *The Johns Hopkins Medical Journal* 121: 1-8, July 1967.

4. Fahey, J.L., Carbone, P.P., Rowe, D. and Bachmann, R.: Plasma cell myeloma with D-myeloma protein (IgD myeloma). *American Journal of Medicine* (In Press).

5. Carbone, P.P., Zipkin, I., Sokoloff, L., Frazier, P., Cook, P. and Mullins, F.: Effect of fluoride on bone in plasma cell myeloma. *Archives of Internal Medicine* 121: 130-140, February 1968.

Serial No. NCI-6075
1. Medicine Branch
2. Solid Tumor Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Kinetics of Cellular Proliferation

Previous Serial Number: None

Principal Investigators: Vincent T. DeVita, Robert Young, Seymour Perry

Other Investigators: Ronald Yankee, Charles Vogel

Cooperating Units: Medicine Branch, Solid Tumor Service, NCI
Acute Leukemia Service, NCI

Man Years:

Total:	2
Professional:	2
Other:	0

Project Description:

Objectives:

1. To obtain information on the kinetics of solid tumor growth in humans and laboratory animals.

2. To attempt to apply such information to the design of therapeutic trials of chemotherapeutic agents for cancer in man.

Methods Employed:

Either intravenous injection as a pulse or continuous infusion of tritiated thymidine has been employed in conjunction with serial biopsies of tumor masses to measure parameters of growth. Single pulse injection can only give an estimate of the labeling index, the number of cells in DNA synthesis at any one instant; continuous infusion can give information about the size of the proliferating pool of cells over the period of time of the infusion. To obtain information about the duration of the S (DNS synthetic phase) local injection with closely spaced serial biopsies have recently been employed.

The tissue obtained by biopsy is processed for radioautography and the parameters mentioned above determined by reading microscopic sections covered with radioautograms.

Number of Patients Studied:

A. Single pulse injections

Three patients - results pending

B. Continuous infusion

One Hodgkin's disease - 67 hour infusion
One melanoma - 24 hour infusion

C. Local tumor injection

Two patients with carcinoma of the breast
Two patients with melanoma
One patient with Hodgkin's disease

Results:

It appears that the usefulness of a single pulse injection is limited because of the long period of time for development of the radioautograph and erratic labeling. It has been possible to calculate the duration of S phase by repeated local injection and serial biopsies, and attempts are underway to obtain this type of information on groups of patients with the same tumor to test the consistency of results.

The use of double label techniques coupled with local injection may provide an even more rapid method of calculating the duration of S phase. Most of the studies are still in the preliminary phase in human solid tumors.

Animal Studies:

A. Leukemia L1210. A study on the cell cycle of leukemia L1210 *in vivo* has been completed and published. The results indicate that the tumor divides rapidly (T_G 12 hours) with 75% of the cycle occupied by S phase (9 hours). The entire pool of cells is proliferating; the results help to explain the chemotherapeutic responses in the tumor system.

B. CDF_1 Mouse Leukocyte Kinetics. In conjunction with the study mentioned under A above, a study has been completed on the kinetics of the mouse leukocyte using intravenous injections of tritiated thymidine.

The results indicate that the cellular kinetics in mouse marrow are different, generally cell transit times are shorter and phases of the cell cycle are shorter. These results indicate that transformation of schedules obtained using L1210 leukemia in the host CDF_1 mouse should allow for known differences in the kinetics of human and mouse marrow.

Honors and Awards: None

Publications:

1. Yankee, R.A., DeVita, V.T. and Perry, S.: The cell cycle of leukemia L1210 cells *in vivo*. *Cancer Research* 27: 2381-2385, 1967.

Serial No. NCI-6076
1. Medicine Branch
2. Solid Tumor Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Physiologic Disposition, Metabolism and Cellular Interaction of Antitumor Agents

A. Cyclophosphamide
B. Imidazole carboxamide
C. Anthramycin methyl ether
D. Amritamycin

Previous Serial Number: None

Principal Investigators: Vincent T. DeVita, Richard Adamson, Paul P. Carbone, Charles L. Vogel, John L. Ziegler

Other Investigator: Charlene Adamson

Cooperating Units: Solid Tumor Service, Medicine Branch, NCI
Laboratory of Chemical Pharmacology, NCI
Lymphoma Treatment Center, Kampala, Uganda

Man Years:

Total:	2
Professional:	2
Other:	0

Project Description:

Objectives:

A. Cyclophosphamide metabolism: Cyclophosphamide is an effective antitumor agent in man, particularly in the therapy of the Burkitt lymphoma in Africa, which may be curable as often as 20% of cases so treated. The drug is extremely active against mouse tumor and has been shown to be metabolized differently by mice; the drug is metabolized to an active form by the animals. The major purpose of this project is to study the metabolism of cyclophosphamide in patients who respond to the drug and compare the results to non-responders; to define the following possible explanations for the difference:

1. Genetic influences on drug metabolism.

   a. Differences between African and American children in the ability to cleave the drug to the active form.

b. Do responders metabolize the drug differently on a genetic basis?

2. Differences in metabolism of the drug in patients initially sensitive but now resistant; is resistance due to failure to metabolize?

3. The ability of tumor tissue itself to cleave the drug. Difference between sensitive and resistant tissue.

4. The ability of other organs, particularly the reticuloendothelial system, to metabolize the drug. Is the difference in response rate between African children and American children with Burkitt-like lymphoma in part due to the hypertrophied R-E system of the former, in association with many infections particularly malaria?

B. Imidazole carboxamide, NSC 82196, is a new derivative of the imidazole series with potential antitumor activity in humans. The drug is available labeled with ^{14}C on the 2C position of the ring. Studies are in progress to determine the physiologic disposition of this compound in animals and man and to identify its metabolic degradation and site of action.

C and D. Early basic pharmacologic information is necessary about drugs to be used in clinical trials against cancer. To this end studies on several new promising antitumor antibiotics, anthramycin methyl ether and amritamycin have been instituted. Specifically the antitumor spectrum in animals, the effects of these drugs on pharmacologic parameters such as: hexabarbital sleeping times, blood pressure, respiration (before and after various autonomic stimulants) and the uptake of tritiated thymidine, uridine and amino acids into cells both sensitive and resistant.

Methods Employed:

Case material: The physiologic disposition and metabolism of ^{14}C labeled cyclophosphamide was studied in 18 patients in Kampala, Uganda, East Africa, from 4 March 1968 to 17 March 1968.

Samples were frozen and shipped to the NCI laboratories in the United States where processing is taking place at present.

Studies with NSC 82196 have not reached the point where studies in man are yet justified. It is anticipated that with the acquisition of more animal data that patients with metastatic malignancies will be studied during their therapy with NSC 82196.

It is felt that studying the pharmacology during therapeutic trials is particularly useful in defining differences between human responders and non-responders.

Laboratory methods: All counting of radioactivity is done by liquid scintillation methods. Tissue distribution studies are performed by tissue solubilization prior to scintillation counting. For determining the radioactivity in plasma and urine due to cyclophosphamide and/or metabolites, the method of Mellet was used, which is based on differential solubilities of each product. Further identification of metabolites and intact drug is done by thin layer chromatography.

Results:

A. Cyclophosphamide: Evaluation of the data in the first 18 patients is in progress. Preliminary results indicate good recovery of the isotope in the urine with a fair proportion as the metabolite. Plasma levels of drug are mostly intact cytoxan with 17% as metabolite by 6 hours. At this junction these results do not appear significantly different than in patients in the United States.

B. Imidazole carboxamide: Excretion, metabolism and tissue distribution of ^{14}C labeled NSC 82196 in mice is currently under study with further studies in dog, monkey and man planned. Thirty to fifty per cent of each oral dose administered to mice is either excreted in the feces or found in residual gastrointestinal contents. Approximately 30% of the drug is excreted in the urine within 24 hours. Recovery of radioactivity from the central nervous system is very low. Sixty per cent of the radioactivity recovered in the urine migrates on radiochromatograms as the specific "transformation product" (NSC 712970) which although purified has not been further identified. The remaining urinary radioactivity is found in a streak extending upwards from the transformation product. This probably represents intact drug converting to transformation product during migration up the plate. Innumerable chromatographic systems have been tried in an effort to eliminate this streaking but without success to date. The drug's instability to pH and temperature changes, its striking light sensitivity, and its relative involubility in most physiologic vehicles, makes the oral dosage form the only one available for clinical studies. From our preliminary studies it would seem that the incomplete and erratic gastrointestinal absorption of this drug may create some problems in establishing safe and predictable doses in the clinic.

C and D. Studies with anthramycin (AMS) have been completed. The drug increases the survival of mice bearing various experimental leukemias. No effects were noted on blood pressure or respiration nor did it alter the response to various autonomic agents. AME normally inhibited the uptake of tritiated uridine in both L1210 and p388 leukemic cells *in vivo*. It also interferred with the uptake of tritiated thymidine in L1210 cells.

Studies with amritamycin are still in progress.

Honors and Awards: None

Publications:

1. Adamson, R.H., Hart, L.G., DeVita, V.T. and Oliverio, V.T.: Antitumor activity and some pharmacologic properties of Anthromycin methyl ether. Cancer Research 28: 343, 1968.

2. Rall, D.P. and DeVita, V.T.: Pharmacologic aspects of the chemotherapy of solid tumors. Pharmacology for Physicians (In Press).

Serial No. NCI-650 (c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Service Radiation Therapy

Previous Serial Number: Same

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigators: A. Robert Kagan, M.D., Mark D. Hafermann, M.D., Robert W. Swain, Dwight W. Glenn, M.S., and Marilyn Glover, R.N.

Cooperating Units: None

Man Years:

Total:	4/6
Professional:	1/2
Other:	1/6

Project Description:

Objectives: To provide high-quality clinical radiotherapy for patients (other than Radiation Branch research patients) at the Clinical Center of the National Institutes of Health. In addition, a consultative service is provided, and a number of patients not receiving radiotherapy are seen on this basis.

Patient Material: Approximately 300 patients were seen in consultation during the twelve month period preceding this report, the considerable majority of these being from the other clinical services of the National Cancer Institute.

Methods Employed: The majority of patients were treated with 2 Mev x-rays and Cobalt teletherapy.

Major Findings: Aside from results on research patients as detailed in other project reports, the response to radiation therapy has been consistent with that reported by others.

Proposed Course: To continue.

Honors and Awards: None

Publications: None

Serial No. NCI-684
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Non-clinical Service Irradiation

Previous Serial Number: Same

Principal Investigator: Robert W. Swain

Other Investigator: Araxie Yacoubian

Cooperating Units: None

Man Years:

Total:	1-1/2
Professional:	1/2
Other	1

Project Description:

Objectives: To provide facilities and to perform irradiation of materials as requested. This includes providing accurate dosimetry and suggestions as to preparation of materials, type of radiation to be used and procedures for irradiation.

Methods Employed: The 3 Mev and 2 Mev Van de Graaff generators for electrons or x-rays, and 250 KVCP x-ray generators, along with operators, are provided and maintained in proper operating condition.

Major Findings: The tables indicate the organizations for which the irradiations were performed, the number of exposures, dose range, and material irradiated. The number of exposures is no indication of the amount of material irradiated. In most cases, during a single exposure with the Van de Graaff generators, as many as 10 samples are irradiated during a single exposure.

Proposed Course: To continue.

Honors and Awards: None

Publications: None

Services Provided on FD Generator
April 1, 1967 through March 31, 1968

Organization	Material Irradiated	Exposures	Exposure Range
C-R	Mice, cells	96	250 - 1600 R
C-LPHY	Protein	88	12 - 28 x 10^6 rads
C-VB	Chambers	11	3 x 10^6 rads
C-LP	Filters	1	4 x 10^6 rads
C-LBGY	---	1	3 x 10^6 rads
D-LM	Animal food	144	4 x 10^6 rads
R-LA	Animal food	11	4 x 10^6 rads
I-LI	Hamsters	3	1500 R
	Total	355	

Services Provided on 250 KVCP Westinghouse Machine
April 1, 1967 through March 31, 1968

Organization	Material Irradiated	Exposures	Exposure Range
C-LPHY	Mice, rabbits	3938	300 - 4000 R
C-R	Mice	1486	95 - 540 R
C-PA	Hamsters, mice	69	300 - 500 R
C-CCNSC	Mice, cells	339	350 - 7000 R
C-VB	Mice, hamsters	65	300 - 600 R
C-LBGY	Mice	427	160 - 425 R
C-M	Mice	9	150 - 200 R
C-SURG	Mice, hamsters	21	400 R
C-I	Cells	27	3000 - 10,000 R
C-MET	Chickens	33	450 - 650 R
C-DE	Mice	161	400 R
C-BGY	Mice, hamsters	7	300 R
C-CP	Mice	44	400 R
C-GL&C	Mice	124	900 R
C-LB	Mice	4	850 R
C-VLL	Mice	9	350 R
C-E	Cells	5	1000 R
C-VC	Mice	13	300 R
A-LPB	Mice	188	500 - 1000 R
A-A&R	Cells	12	2500 - 5000 R
BS-LVI	Cells	12	3000 R
CC-BB(?)	Mice	4	300 R

CC-CP	Mice		24	550 - 950 R
D-HG	Cells		1	1000 R
I-LGAR	Mice		33	500 R
I-LBV	Mice		20	500 - 900 R
I-LCI	Cells		10	5000 - 10,000 R
		Total	7085	

Serial No. NCI-956 (c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Role of Total Body Irradiation in the Treatment of Generalized Lymphoma and Leukemia

Previous Serial Number: Same

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigators: Robert W. Swain, Dwight W. Glenn, M.S., and A. Robert Kagan, M.D.

Cooperating Units: None

Man Years:

Total:	4/6
Professional:	1/2
Other:	1/6

Project Description:

Objectives: The objective of this study is to evaluate fractionated total body irradiation in the treatment of leukemias and generalized lymphomas.

Patient Material: A total of 75 patients have entered the study to date. These have included patients with diagnoses of chronic lymphatic leukemia, generalized lymphosarcoma, mycosis fungoides, and acute leukemia.

Methods Employed: Total body irradiation with Co^{60} gamma rays and 2 Mev x-rays have been administered using fractionated schedules. Hematopoietic effects as well as other clinical parameters have been carefully evaluated. The dosimetry has received special attention and both phantom and *in vivo* measurements have been undertaken. Tumor response has been carefully documented and in some cases, maintenance treatment has been instituted following an initial intensive course of irradiation.

Major Findings: Objective responses have been observed. Complete clinical remissions have been achieved in patients with lymphosarcoma and excellent hematologic improvements have occurred in patients with chronic lymphocytic leukemia.

Proposed Course: Accession of patients into the study will continue.

Honors and Awards: None

Publications:

1. Johnson, R.E., Foley, H.T., Swain, R.W., O'Conor, G.T.: Treatment of Lymphosarcoma with Fractionated Total Body Irradiation, *Cancer* 20: 482-485, 1967.
2. Johnson, R.E., Kagan, A.R., Gralnick, H.R., and Fass, L., Radiation-Induced Remissions in Chronic Lymphocytic Leukemia, *Cancer* 20: 1382-1387, 1967.
3. Johnson, R.E., Total Body Irradiation with Fractionated Dosage Schedules, Proceedings of the Third Annual San Francisco Cancer Symposium (In Press).

Serial No. NCI-958 (c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Radiotherapeutic Management of Hodgkin's Disease

Previous Serial Number: Same

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigators: Dwight W. Glenn, M.S., Robert W. Swain, Marilyn Glover, R.N., A. Robert Kagan, M.D., and Mark D. Hafermann, M.D.

Man Years:

Total:	7/6
Professional:	6/6
Other:	1/6

Project Description:

Objectives: The investigation of (a) the value of certain diagnostic techniques (e.g., lymphangiogram) in guiding treatment rationale, (b) to compare various treatment regimens in controlled clinical trials for localized Hodgkin's disease, (c) and investigate the role of intensive radiotherapy in the treatment of patients with generalized involvement.

Patient Material: To date, a total of 100 patients with previously untreated Hodgkin's disease have been entered into the study.

Methods Employed: Prior to initiation of treatment, a detailed and exhaustive diagnostic evaluation is carried out with dual purpose. First is the necessity of accurately establishing the extent of disease within the limits of the most sophisticated current diagnostic means. Secondly, the opportunity is provided for the evaluation of the merit of the available diagnostic tests in guiding decisions as to optimal therapy. This latter is being undertaken by the randomization of patients into one of several treatment programs, depending on the clinical extent of disease. Likewise, these controlled trials will validly compare the merits of limited versus extended radiotherapy in the treatment of Hodgkin's disease.

Major Findings: It has been determined that radical extended radiotherapy is tolerated both systemically and hematopoietically. Complete clinical remissions have not only been achieved regularly in patients with localized clinical involvement but also in some patients with advanced disease when initially admitted for evaluation.

Proposed Course: Limited accession of patients into the study will continue.

Honors and Awards: None

Publications:

1. Johnson, R.E., The Radiotherapy of Hodgkin's Disease (In Proceedings of the N.I.H. Combined Clinical Staff Conference on Hodgkin's Disease) Ann. Int. Med. 67: 424-442, 1967.
2. Glenn, D.W., Faw, F.L., Kagan, A.R., and Johnson, R.E., Field Separation in Multiple Portal Radiation Therapy, Amer. J. Roent. 102: 199-206, 1968.
3. Johnson, R.E., and Cook, P.L., Hodgkin's Disease: The Negative Lymphogram in Guiding Radiotherapy, Amer. J. Roent (In Press).
4. Johnson, R.E., Comparison of Recurrence Patterns in Human Lymphoma and Experimental Leukemia, Cancer (In Press).

Serial No. NCI-961
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Radiation Effects on Mammalian Tumor Cells

Previous Serial Number: Same

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigator: Walter G. Hardy

Man Years:

Total: 12/12
Professional: 1/12
Other 11/12

Cooperating Units: None

Project Description:

Objectives: To study the effect of various forms of ionizing radiations on the biological characteristics of mammalian tumor cells. The objects of the studies are to increase understanding of radiation effects on tumor cells and to investigate the potential effects of these induced changes on radiosensitivity.

Methods Employed: The L1210 murine leukemia system is used and techniques have been devised for the study of radiation effects on both single cells and cell populations.

Major Findings: These studies are in a preliminary phase, and much of the effort continued to be directed toward quantitating and increasing the sensitivity of the model system which is being developed.

Proposed Course: To continue.

Honors and Awards: None

Publications:

1. Johnson, R.E., Survival Curve Characteristics of Drug Resistant Murine Leukemia, Radiology 88: 988-992, 1967.

2. Johnson, R.E., Comparison of Recurrence Patterns in Human Lymphoma and Experimental Leukemia, Cancer (In Press).

Serial No. NCI-962
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Development of a Transmitted Radiation Dosimeter for Clinical Studies

Previous Serial Number: Same

Principal Investigator: Robert W. Swain

Other Investigators: Ralph E. Johnson, M.D., and Frederick Faw, M.S.

Man Years:

Total:	1/6
Professional:	1/12
Other:	1/12

Project Description:

Objectives: To develop a device for the measurement of transmitted radiation doses through various absorbing media. Phantom studies have been carried out, and transmission measurements on patients are being undertaken. This will provide information which will make possible dosimetry corrections required for inhomogeneities in absorption produced by variations in body densities (e.g., bone, lung).

Patient Material: A series of patients are being evaluated. The studies indicate this method of correcting depth doses for body inhomogeneities will be more accurate than currently available tables empirically constructed.

Methods Employed: A well-colliminated scanning device has been constructed to measure the transmitted dose from a Co^{60} or Cs^{137} source. The time required for registration of a present number of counts is translated into thickness in unit density.

Major Findings: Phantom measurements have been carried out for calibration purposes and clinical investigations have been undertaken.

Proposed Course: To continue with clinical evaluation of the transmission dosimeter

Honors and Awards: None

Publications: None

Serial No. NCI-6050
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Computer Dosimetry for Radiation Therapy

Previous Serial Number: None

Principal Investigator: Frederick L. Faw, M.S.

Other Investigators: Dwight W. Glenn, M.S.

Man Years:

Total	12/12
Professional:	12/12

Project Description:

Objectives: To evaluate the potential of computers for the rapid and more accurate performance of complicated dosimetry calculations required in clinical radiation therapy.

Methods Employed: A console computer is being programmed for future application in clinical radiotherapy.

Major Findings: Preliminary experience has indicated the great possibilities which exist in the use of computers for facilitating the solving of complex dosimetry problems which frequently present themselves in radiation therapy.

Proposed Course: To continue.

Honors and Awards: None

Publications: None

Serial No. NCI-6051
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Protection against Radiation Nephritis with Renal Artery Infusion of Epinephrine

Previous Serial Number: None

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigators: Mark D. Hafermann, M.D.

Man Years:

Total:	12/12
Professional:	6/12
Other:	6/12

Project Description:

Objectives: The concept involves the production of renal hypoxia during administration of radiotherapy by the intra-arterial infusion of epinephrine. It is hoped that radiation damage to the normal kidney parenchyma will be prevented by creating a state of transient ischemia.

Methods Employed: An indwelling arterial catheter is placed in the renal artery via percutaneous catheterization. The patency of the catheter is maintained by a continuous infusion of heparinized saline and during daily irradiation, a dilute epinephrine solution is administered.

Major Findings: Physiologic studies have been completed, including arteriography and renography, demonstrating that virtually complete ischemia can be accomplished in the kidney with tolerated doses of epinephrine. This is currently being applied to patients with advanced cancer in the upper abdomen to evaluate the potential protective effect against the development of radiation nephritis.

Proposed Course: To continue.

Honors and Awards: None

Publications:

1. Steckel, R.J., MacLowry, J.D., Holland, J.M., Paulson, D.F., and Johnson, R.E., Radiation Protection of the Canine Kidney by Epinephrine Infusion, Radiology 89: 332-335, 1967.

2. Johnson, R.E., Doppman, J.L., Harbert, J.C., Steckel, R.J., and MacLowry, J.D., Prevention of Radiation Nephritis with Renal Artery Infusion of Vasoconstrictive Agents, Radiology (In Press).

SUMMARY REPORT

CANCER CHEMOTHERAPY NATIONAL SERVICE CENTER

CHEMOTHERAPY
NATIONAL CANCER INSTITUTE

July 1, 1967 -- June 30, 1968

Introduction

During the past year, the Cancer Chemotherapy National Service Center continued to pursue aggressively its primary task of searching for and developing new and better potential antitumor agents, as well as developing new and better methods of drug selection and prediction of optimal drug schedules. In terms of the new linear array developed during the past fiscal year, these tasks represent the major portion of Stage I, Stage II, Stage III b and c, Stage IV b and c, as well as the continuing production and formulation activities running in parallel with the rest of the linear array from Stage II onward.

The program is carried out primarily under contracts with pharmaceutical and chemical companies, research institutes, and academic institutions; also included are the laboratory operations carried out within a contract at Microbiological Associates, Inc. under direct staff supervision and collaboration. The activities depend heavily on additional collaboration with research grantees, other independent investigators throughout the world, and hundreds of industrial companies not involved in contracts, and especially on the invaluable collaboration with staff in other portions of the Chemotherapy Program and the National Cancer Institute.

Contracts totaling about $16,000,000 are currently in effect in the following areas:

1. Synthesis of new chemical agents
2. Procurement and resynthesis of bulk chemical and drugs for preclinical and clinical evaluation
3. Development of new agents from fermentation products
4. Development of new agents from plant and other natural products
5. Development and procurement of clinical dose forms for clinical trial
6. Quality control for bulk and formulated drugs
7. Primary and other screening of new materials
8. Bioassays, both _in vivo_ and _in vitro_, to monitor the purification of fermentation and natural products

9. Study of optimal drug routes and regimens

10. Development of new and improved laboratory methods for evaluating antitumor agents

11. Mechanisms of drug action and resistance

12. Animal procurement and disease control

13. Material resources

14. Procurement of radiolabeled drugs for clinical pharmacologic and experimental studies

15. Development of drugs for the treatment of bacterial, fungal and viral infections in cancer patients

Staff and Organization

The CCNSC laboratory and contract activities during FY 1968 were carried out by a staff of 60: 17 senior professional (GS-13 or higher), 20 junior professional, one guest worker, and the remainder clerical and secretarial. The present organization is presented below.

Cancer Chemotherapy National Service Center

Associate Scientific Director and Chief, CCNSC - Saul A. Schepartz

Associate Chief for Laboratory Research - Abraham Goldin
Mammalian Genetics and Animal Production Section - Samuel M. Poiley, Head

Drug Development Branch - Harry B. Wood, Jr., Chief

Chemical & Drug Procurement Section - Robert R. Engle, Head
Chemical & Drug Records Section - Robert B. Ing, Head
Clinical Drug Distribution Section - J. Paul Davignon, Head
Natural Products Section - Jonathan L. Hartwell, Head

Drug Evaluation Branch - John M. Venditti, Chief

Biochemistry Section - Florence R. White, Head
Immunotherapy and Immune Response Modifiers Section - John P. Glynn, Head
Screening Section - Betty J. Abbott, Head

Major Accomplishments

Stage I - Selection of Agents for Screening

The past year has seen a continuation of the review and evaluation of the nature of the input to the screen. An analysis of CCNSC synthetic compounds indicates that only a small percentage of published types of compounds, based on ring structures, have been submitted to the program (6.4%). With very few exceptions, large numbers of compounds in a given class have been evaluated only if that class included one or more active compounds. Thus, it is difficult to conclude that any given class has been evaluated adequately and found to be devoid of antitumor activity. To the extent that data exist, however, the CCNSC staff attempts to utilize such data and direct the emphasis of the program into those areas in which a high of probability of activity is expected, and away from those areas that appear to be adequately studied. Thus, a de-emphasis in alkylating agents, other than those in the cytoxan and nitrosourea areas, has been attempted for several years.

Increased emphasis has been placed on the acquisition and development of nucleosides and heterocyclic compounds. This change in emphasis is clearly demonstrable in the contract synthesis program. Since only about 10% of synthetic compounds submitted to the program derive from contract laboratories, however, these shifts in the contract program do not necessarily reflect overall shifts in submittals to the screen. Research programs funded under National Institutes of Health grants have tended to move in the same direction as our contract programs through our staff's direct contact with suppliers and close association with the executive secretaries of the medicinal chemistry study sections. There are considerable lags in the shift of grant programs from one area to another, however, and it is not quite as evident that significant shifts away from alkylating agents into other areas have occurred. Efforts will continue, however, to place the greatest emphasis in those areas that appear to be most worthy of exploitation.

With regard to natural products, a considerable effort has been made during the past year to broaden the base of accessions of natural products beyond the higher plants that have occupied almost the entire natural product program thus far. Late in FY 1967 two contracts for the production and extraction of algae cultures were implemented, as well as a contract for the collection and extraction of marine invertebrates. One of the algae contracts will be continuing and it is hoped that within another year or two, a sufficient number of cultures will be sampled to determine if this type of material possesses antitumor activity. It is hoped that additional efforts in the marine invertebrate field will be possible. Prior information, regarding the activity of clam extracts and sponges, suggests that the marine area may be a valuable source of new materials. During the past year a small number of insects were procured, extracted and submitted to the screen. Two materials recently showed confirmed activity and hopefully will be undergoing further extraction and purification studies during the next year. This development is highly encouraging and it is hoped that additional efforts in the area of insects may be possible.

During the period of this report, materials continued to be obtained through a variety of mechanisms, including direct purchase, synthesis contracts, natural product development contracts and the voluntary submission of a wide variety of materials by grantees and other investigators throughout the world. Listed below are the numbers of materials submitted during calendar 1967 compared with comparable figures for 1966. In order to provide exact numbers for this report, it is necessary to use the calendar year rather than the fiscal year. It should be noted that a major increase in plant products accounts for a net increase in the total number of materials submitted.

	1967	1966
Synthetics	7,413	8,008
Fermentation Products	4,982	4,284
Crude Plant Products	6,253]	3,620
Animal Products	446]	
Total	19,094	15,912

Stage II

Determination of Antitumor Activity of New Agents

During FY 1968, primary screening consisted of the L1210 tumor system in mice and the Walker 256 (IM) in rats as described in the last annual report. In December 1967, a detailed review was made of the screen and of the possible need to change the systems involved. It was agreed that the L1210 screen, both Day 2 only treatment and treatment days 1-9, should be continued, since it is the system largely responsible for the development of new effective agents. It was agreed that the Walker 256 tumor system should be phased out and replaced by one or more other tumor systems. There were several factors leading to this decision: (1) The known active agents that are effective in the Walker 256 system and not effective in L1210 tend to be useful in a rather restricted area of human tumors, largely lymphomas and chronic leukemia. (2) A substantial number of new agents are currently in process toward clinical trial based on activity in the Walker 256 system. In addition, a significant number of additional agents currently in the process of purification will be forthcoming in the next few years. (3) The Walker system is a relatively rapidly growing and responsive system. Since the L1210 system is also a rapidly growing system we do not have in the screen a slowing proliferating tumor system that is relatively non-responsive and may be of greater significance in selecting materials for the large solid tumors that represent the major clinical problem today.

Much of scientific basis for the switch to a "slowly growing" tumor, or one with a small proliferative pool, results from the extensive efforts of the contract program at Southern Research Institute in collaboration with clinical and other investigators at the National Cancer Institute and elsewhere. The extensive probing for new approaches, carried out primarily at Southern Research Institute, Arthur D. Little, and in the staff-directed program at Microbiological Associates, Inc., is essential to provide a continuing supply of new experimental ideas.

Since the decision made in December 1967, efforts have been made to cut back the screening in the Walker 256 system. Practical problems relating to animal supply and the lack of a definitely evaluated tumor system to take the place of the Walker 256 have tended to inhibit the implementation of this change. Four tumor systems are currently under evaluation as potential screens. These are the Lewis Lung Carcinoma, Hepatoma 129, B-16 melanoma, and the

Harding-Passey melanoma. These were initially selected as candidates on the basis of published or other data suggesting that they are slowing proliferating tumors that are relatively non-responsive. It is hoped that further studies in the next few months will enable us to select one or more of these tumors for addition to the screen in accordance with Stage II, Step 2.

In regard to the direct progression of the materials through Stage II of the linear array, 180 synthetic compounds showed confirmed activity during the calendar year 1967 and reached the point where further testing would be needed in order to determine whether they are of sufficient interest and are otherwise qualified to progress to toxicology and eventual clinical trial. During this same period of time, 234 crude fermentation, plant, and animal products demonstrated confirmed activity and began the recycling process toward eventual purification and definitive evaluation. Thus, about 2% of the natural products and 2.5% of the synthetic compounds moved through Stage II of the linear array and required further work. An additional significant discard rate occurs in the course of making the decision at the end of Stage II. Thus, during calendar year 1967, eight synthetic compounds, four plant products, and two fermentation products were placed in Step IVA and, therefore, scheduled for preclinical toxicology (Stage III). A further decrease occurs in the course of developing new agents for clinical trial. This is evidenced by the fact that during calendar year 1967, only eight IND applications were filed with the Food and Drug Administration. Included in this group were only five new drugs:

1. NSC-11905 - Lapachol
2. NSC-85998 - Streptozotocin
3. NSC-75520 - Uridine, -deoxy-5-(trifluoromethyl)- (F3TDR)
4. NSC-82196 - Imidazole-4(or 5)-carboxamide, 5(or 4)-[3,3-bis(2-chloroethyl)-1-triazeno]-
5. NSC-88536E - Androst-4-en-3-one, 17β-hydroxy-7β,17-dimethyl- (Testosterone, 7β,17-dimethyl-)

Thus, an average of about one material out of 2000 reaches clinical trial. In carrying out its functions in moving drugs in and through Stages I and II of the linear array, the CCNSC, through its screening laboratories, carried out about 180,000 primary screening and related tests. For these and other experimental tumor studies, about 3 million experimental animals were produced or procured. The changing requirements for screening systems that have occurred over the last several years have placed a severe strain on the screening laboratories and the animal procurement program because of the exceedingly complicated logistics involved. It is a tribute to the efforts of the staff that these frequent changes have been carried out as efficiently as they have.

Future Efforts

With regard to the submission of materials to the screen, it is expected that efforts will be continued toward the procurement of new types of natural products, representing a wide variety of Phyla and geographical sources, within the limitations of available funds. Efforts will be continued to analyze, from the standpoint of structure-activity relationships, selected areas of synthetic compounds in order to draw conclusions regarding areas to emphasize and de-emphasize. The major effort in screening research and development will be the evaluation of the four tumor systems mentioned early, in order to select the system or systems most suitable for addition to the screen. Such a system should have a relatively small proliferative pool resulting in a relatively long doubling time, it should be insensitive to most of the agents effective against L1210; and it should be feasible to carry out on a relatively large scale.

Efforts will, of course, continue to supply the materials needed for this and other parts of the program including adequate numbers of healthy animals, quantities of bulk and formulated drugs as needed, and other supporting services.

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1. Office of Associate Scientific Director and Chief, CCNSC
2. --
3. Wiscon Building - Kenwood Laboratories Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Chemotherapy research including fundamental biological and biochemical investigations of antitumor agents

Previous Serial Number: Same

Principal Investigator: A. Goldin

Co-Principal Investigators: W. D. DeWys, S. R. Humphreys, V. A. Masilungan, A. W. Schrecker, S. Tsukagoshi, J. M. Venditti

Other Investigators: D. Abraham, J.A.R. Mead

Cooperating Units: M. Friedkin and staff at Tufts University Medical School, Department of Pharmacology; N. Kaplan and G. Sato, Graduate Department of Biochemistry, Brandeis University; F. M. Huennekens, D. Visser and D. Jacobson, Scripps Clinic and Research Foundation; I. Wodinsky and C. Kensler, A. D. Little, Inc.; J. M. Venditti and staff, Drug Evaluation Branch, NCI; J. P. Glynn, Drug Evaluation Branch, NCI; H. B. Wood, Jr. and R. R. Engle, Drug Development Branch, NCI; R. Johnson, Radiation Branch, NCI; A. DiMarco, Farmitalia, Milan, Italy; I. Kline, S. Vadlamudi, V. Waravdekar, Microbiological Associates, Inc.

Man Years

Total: 7 1/4
Professional: 5 1/4
Others: 2

Project Description:

Objectives:

To conduct fundamental investigations of potential and established clinical cancer chemotherapeutic agents; to obtain biological and biochemical knowledge concerning chemical agents capable of affecting malignant growth; to determine means for improvement of therapy with existing drugs; to investigate the influence of structural modification of drugs on the mechanism of antitumor action; to provide basic information pertaining to the clinical use of such agents; to study specific problems encountered during the use of these agents in the clinic.

Specific objectives include:

I. Investigations of primary and secondary screening procedures and methodology employed in drug evaluation.

  A. Investigation of characteristics of ongoing screens and of new screening systems which may broaden the base of predictability of drugs for clinical activity. Emphasis is placed on predictability systems for clinically useful compounds which were not predicted by the standard screen and systems for the selection of new and novel structures which may have potential in clinical chemotherapy. Naturally resistant tumors with a long doubling time are under consideration.

II. Investigation of the influence of drugs on tumor cells.

  A. Detailed investigation of the mode of action of drugs including purine and pyrimidine antagonists, folic acid antagonists, alkylating agents, etc.

    1. Determination of the extent and duration of drug action.

    2. Study of the influence of drug on the mitotic cycle.

    3. Study of the lethal action on tumor cells.

    4. Investigation of problems relating to drug resistance.

    5. The influence of chemical agents on metastasis and tumor cell sequestration including intracranial involvement.

    6. Studies of drug transport.

    7. Investigation of the sites of action of drugs.

    8. Study of the influence of drugs on the hematopoietic and tissue components of the host in relation to antitumor activity.

  B. Investigation of factors which may influence drug action in relation to improvement of therapy.

III. Study of the influence of drugs on tumor-host interactions.

  A. Investigation of the basis for drug specificity.

        1. Exploration of the influence of novel schedules. Investigation of the underlying basis for drug scheduling and combination chemotherapy.
        2. Detailed studies in inhibition analysis.
            a. Determination of metabolite-antimetabolite interrelationships for folic acid congeners, purine antagonists, etc.
            b. Studies on the sparing action of drugs.
            c. Investigation of the inhibition of de novo synthesis, lethal synthesis, feedback inhibition, etc.
            d. Investigation of collateral sensitivity as a phenomenon which may lead to improved therapy.
    B. Exploration of means for augmentation of the host contribution to chemotherapy.
    C. Chemotherapeutic investigations on tumor cell kinetics with a view to the total eradication of the tumor cell population in the host.

IV. Antiviral chemotherapy.

    A. Investigation of the effect of chemical agents and their mechanism of action on leukemogenic and solid tumor-inducing viruses.
    B. Study of the action of drugs on virus-induced tumors.
    C. Biological and biochemical investigation of the interaction of viruses and cells in relation to drug action.

V. Exploration of new and potentially useful biological and biochemical methodology for prediction, evaluation and improvement of drug therapy. Attention will be focused on studies of remission induction and remission maintenance.

This program is planned so that it will be highly responsive to the needs of the preclinical and clinical chemotherapy program of the Cancer Institute.

The Kenwood Laboratories of Microbiological Associates staffed by Microbiological Associates professional and technical personnel serve as a collaborative laboratory resource for the research staff of this program. The chemotherapy research program collaborates closely with the ongoing programs in drug evaluation, immunology, organic chemistry and pharmacology. The NCI professional staff participated in a day to day advisory capacity in the execution and evaluation of the experimental programs. This collaborative laboratory program of NCI scientists serves as a focal point for the interdigitation of chemotherapy research including the CCNSC screening, developmental and basic chemotherapeutic investigations with collaborating CCNSC contractors, and clinical programs of NCI and other institutions. It maintains close relationships with medical schools, pharmaceutical houses, university and research institutes, both in the United States and abroad in areas pertinent to chemotherapy.

Methods Employed: Standard laboratory procedures were employed.

Major Findings:

I. The Relationship of Structure to Antitumor Activity

In continuation of the overall program of the Scientific Director for Chemotherapy, a series of retrospective analyses are in progress on the structure-activity relationships of classes of drugs of clinical interest. In last year's annual report, mention was made of a detailed structure activity study on "Relationship of Structure to Antitumor Activity in Compounds Related to Folic Acid" prepared by J.A.R. Mead, H. B. Wood, Jr., and A. Goldin. This report is currently being readied for publication.

During the year a second study was conducted on the "Relationship of Structure to Antitumor Activity for Purine and Pyrimidine Nucleosides" by A. Goldin, H. B. Wood, Jr., and R. R. Engle. This report was prepared initially for a meeting of the Scientific Director for Chemotherapy in May 1967 on purine and pyrimidine nucleosides. A full scale report is currently being readied for publication. In the report the structure activity relationships are reviewed for the purine and pyrimidine nucleoside derivatives subjected to testing in the screening program of the Cancer Chemotherapy National Service Center. Data are presented on 648 compounds in 36 structural groupings.

The test systems include leukemia L1210, Walker carcinosarcoma 256, adenocarcinoma 755, sarcoma 180 and Lewis lung tumor in mice, plus the KB tissue culture screen. Also indicated is the clinical status of the nucleoside derivatives. Twenty-one nucleoside derivatives are in the clinical category. Sixteen of these have shown clinical activity or are being actively processed for the clinic and were active in the leukemia L1210 system. One of the compounds active in the clinic was inactive in the leukemia L1210 system but was active against KB in tissue culture. One additional compound active in the clinic was inactive in the screening systems. Three compounds listed as inactive in the clinic or too toxic for clinical use were active only in the KB system.

One hundred twenty-nine additional compounds for which to date there has been no clear clinical interest were found to be active in one or more of the experimental test systems. Included were 35 compounds active against leukemia L1210, one compound active against Walker 256, 69 compounds active against carcinoma 755, one compound active against Lewis lung tumor, and 23 compounds active against KB cells in tissue culture. These compounds appeared in 20 of the 36 structural groupings, indicative of the wide range of activity of purine and pyrimidine nucleoside congeners.

Compounds active against leukemia L1210 were found in the groupings: uracil nucleosides, cytosine nucleosides, azacytidine nucleosides, hypoxanthine nucleosides, 6-mercaptopurine nucleosides, 6-chloropurine nucleosides, adenine nucleosides, thioguanine nucleosides and 7 deaza nucleosides. Active compounds were obtained with ribose, deoxyribose, arabinose, glucopyranose, tetrahydrofuran and tetrahydropyran.

The structure-antitumor relationships for the compounds listed in this report emphasize the desirability for the further investigation and possible introduction into the clinic of additional ones of the active nucleoside derivatives. The analysis also indicates that simple structural alterations in this class of compounds may lead to wide alteration in biological and antitumor activity suggesting the importance of additional stress in the synthesis of new congeners. It is considered that this report may provide a background for additional synthesis and investigation of the purine and pyrimidine nucleosides.

II. Metabolite-Antimetabolite Relationships

Antimetabolites have a firm place in the chemotherapy of neoplasia but the identification of corresponding metabolites and their use in effective control of desirable toxicity for the host has been largely unexploited. A notable therapeutic advantage was obtained in the chemotherapy of leukemia L1210 on the delayed administration of citrovorum factor following methotrexate. Although not tested definitively in the clinic, this observation has stimulated additional interest in the elucidation of metabolite-antimetabolite relationships for chemotherapy.

1. 5-Azacytidine (S. Vadlamudi and A. Goldin)

5-Azacytidine is an analog of cytidine which has been reported to have antibacterial and antitumor properties. Its inhibitory action has been attributed to a block in the synthesis of pyrimidine components of nucleic acid. 5-Azacytidine was shown to have a definite and reproducible inhibitory effect against leukemia L1210 in mice as reflected in increases in survival time ranging in the area of 100% over controls. Bioassay of the spleen and bone marrow of treated animals demonstrated that 5-azacytidine was markedly effective in reducing the number of leukemic cells.

An initial series of experiments was conducted in which it was demonstrated that simultaneous administration of cytidine was capable of blocking both the antileukemic action of 5-azacytidine and its toxicity for the host. There has been some indication that an actual therapeutic advantage might be obtained in the treatment of leukemia L1210 on the employment of the combination of cytidine plus 5-azacytidine and this is under current investigation. It will be of interest to determine whether with delayed administration of cytidine protection might be afforded for the host with retention of antileukemic effectiveness.

In contrast to cytidine, deoxycytidine was only minimally effective in blocking the antileukemic and toxic actions of 5-azacytidine.

2. Folic Acid Antagonists

Collaborative studies are being continued with J.A.R. Mead and M. Friedkin on tetrahydrohomofolate, dihydrohomofolate and homofolate in the leukemia L1210 system. The clinical interest in these novel congeners of folic acid is warranted by the difference in mechanism of action of these drugs as contrasted with the standard antagonist, methotrexate.

III. Mechanism of Action of Cytosine Arabinoside. (A. W. Schrecker)

1. Nucleoside and Nucleoside Kinase Activities in Leukemia L1210 in Relation to Resistance to Cytosine Arabinoside.

A study was made of the levels of kinase (phosphotransference) activities in the high speed supernatant fraction of ascites cell extracts that were harvested from leukemia L1210 and from a subline rendered resistant to cytosine arabinoside (ara-C).

Of the pyrimidine nucleoside kinases, both ara-C kinase and deoxycytidine kinase were reduced by over 95% in the resistant subline. This reduction was due to an actual reduction in the amount of enzyme present. The presence of an inhibitor of the ara-C-deoxycytidine kinase in the resistant cells was excluded by an experiment in which extracts of sensitive and resistant cells were mixed and the kinase activities found to be additive. The possibility that the resistant cells contained an increased amount of 5'-nucleotidase, which would hydrolyze the phosphorylated product, was also excluded: 5'-nucleotidase activity, measured with ara-CMP-^{3}H (tritiated ara-C 5'-monophosphate), was identical in the sensitive and resistant subline. This 5'-nucleotidase was inhibited 90% by 15mM sodium fluoride.

All the other pyrimidine nucleosides (cytidine, deoxythymidine, deoxyuridine) were phosphorylated to the same extent in both sublines. This result suggests that ara-C kinase and deoxycytidine kinase are identical. This identity was confirmed by kinetic studies which showed that deoxycytidine inhibited competitively ($K_m/K_i = 40$) the phosphorylation of ara-C. Deoxycytidine triphosphate, a known feed-back inhibitor of deoxycytidine kinase, similarly inhibited ara-C kinase, with the same K_m/K_i ratio.

Since Sugino (unpublished results) has shown that deoxycytidine kinase in calf thymus is identical with deoxyguanosine kinase, several purine nucleoside kinases were also investigated. Guanosine, deoxyadenosine and adenosine kinase activities were essentially identical in L1210 and the ara-C-resistant subline. Deoxyguanosine kinase activity, however, was decreased by 50-70% in the resistant subline. This finding showed that the ara-C-deoxycytidine kinase of L1210 also mediates the phosphorylation of deoxyguanosine. (The methods used in measuring kinase activities for the pyrimidine nucleosides were found not to be applicable when guanosine or deoxyguanosine was the substrate, in spite of published reports. A thin-layer chromotography method was developed, which allows accurate assays.)

Several nucleotide kinases were also studied, including dCMP, CMP, ara-CMP, and dCDP kinase. No differences in activity were found between the sensitive and the resistant subline.

2. Studies on Ribonucleotide Reductase in Ascites Cells. (In collaboration with Drs. Frank M. Huennekens, Donald Visser and Donald Jacobson)

Attempts were made to demonstrate ribonucleotide reductase activity in extracts of leukemia L1210 and of Ehrlich ascites carcinoma. So far, no clear-cut activity has been demonstrated. In a number of experiments, an apparent conversion of CDP to dCDP was observed. However, the activity found was spurious and resulted from an artifact in the analytical method: in the degradation of the diphosphates to the monophosphates, CDP-choline, which was formed in a side reaction, was not completely hydrolyzed. Subsequently, A. Schrecker found that in the paper and thin-layer chromatographic systems commonly used, CDP-choline and dCMP have almost identical R_F values. For this reason, an alternative method for the ribonucleotide reductase assay was developed: the reaction mixture is treated with alkaline phosphatase, which hydrolyzes the phosphate esters to the nucleosides. Ribo- and deoxyribonucleosides are then separated very efficiently on polyethyleneimine cellulose thin layers in the borate form. This new method of separation was developed by A. Schrecker recently; it has now been shown to be the method of choice in ribonucleotide reductase assays (carried out with bacterial extracts) when GTP or ATP are the substrates.

IV. Studies with Nicotinamide Adenine Dinucleotide Glycohydrolase (NADase) of Normal and Leukemic Mouse Tissues. (S. Tsukagoshi, M. Kao and A. Goldin)

An approximately 15-30% increase of NADase activity was found in the spleen of leukemic and normal mice at 48 hours after single or daily treatment with BCNU or cyclophosphamide. However, this rise was transitory and was followed by a decrease in spleen NADase activity, reaching a nadir in 8-11 days after initiation of therapy. With BCNU there was also a decrease in NADase in the brain, lung, liver and kidney following treatment. Cyclophosphamide failed to decrease NADase activity in the brain, liver and kidney but there was a slight decrease in the lung at 11 days after treatment. In general, recovery from inhibition of NADase activity was slow and in some instances, such as for the lung of BCNU treated mice, complete recovery did not occur by the end of the observation period.

The observation that BCNU inhibited brain NADase activity while cyclophosphamide did not is in agreement with previous observations that BCNU is effective against meningeal leukemia whereas cyclophosphamide is essentially ineffective. It is suggested that inhibition of brain NADase activity may be employed as an ancillary biochemical parameter in the determination of drug penetration and reactivity.

Both clinical and experimental studies have indicated that delayed and prolonged toxicity may occur as a result of BCNU treatment. It is of interest that a similar delayed and prolonged suppression of NADase activity has been observed in these experiments.

V. Drug Evaluation Systems

The chemotherapy program was represented at the Vth International Congress for Chemotherapy and the European Cancer Congress, both held in Vienna, Austria. At the Vth International Congress for Chemotherapy papers were presented at a Symposium on Test Models and a Symposium on Dose Schedules and Combinations.

A. Test Models for Evaluating the Chemotherapeutic Effectiveness of Drugs. (A. Goldin, S. Vadlamudi, I. Kline, M. Gang and J. M. Venditti)

An analysis was presented of test models which may be employed for expressing drug effectiveness against tumor which take into account the limiting drug toxicity for the host. The systems discussed included (a) tumor growth as an index of drug effectiveness, (b) survival time of tumor-bearing mice as the index of drug effectiveness, (c) total eradication of the tumor as the therapeutic endpoint, (d) the various bioassay techniques including the spleen colony assay as a means for the study of cell cycle sensitivity and tumor cell kinetics, (e) the more complex model in which a tumorigenic virus is the etiologic agent so that account must be taken of the tetrad of host, tumor, virus and drug. The systems are geared to detect drugs which may be active in the clinic and to determine means for the improvement of therapy with known chemotherapeutic agents.

B. Factors Influencing the Therapeutic Effectiveness of Antitumor Agents. (A. Goldin, J. M. Venditti, I. Kline, M. Gang and V. S. Waravdekar)

Attention was focused on the means for augmenting the therapeutic effectiveness of chemotherapeutic agents. This may be accomplished by (a) increasing the toxicity of the drug for the tumor cells relative to the cells of the host or (b) by diminishing the toxicity of the drug for the host to a greater extent than the toxicity for the tumor cells. The antitumor effectiveness of a drug is not a fixed property of the drug. Rather, the potential range of effectiveness of the drug may be realized by appropriate manipulation of the drug, based on specific knowledge pertaining to its mechanism of action with respect to the host and tumor cells. Systems for drug manipulation include:

1. Schedules of administration

    a. Maintenance of blood and tissue levels of drug until all of the tumor cells have been reached and been destroyed in the sensitive phase of their mitotic cycle. In this approach the objective is to destroy the tumor cells as rapidly as possible employing massive doses of the chemotherapeutic agent. For agents which are cell cycle dependent it may be necessary to employ massive infusions extended over a more protracted period of time.

b. In the systems where advantage is to be taken of the differential rate of recovery of the host relative to the rate of recovery of the tumor cell population, the therapy may be administered intermittently in order to provide the host an opportunity to recover so that it can receive additional treatment. In this type of system it is important that the second and subsequent treatments be administered at a time when the critical host cells have recovered fully but the tumor cell population has not regenerated to its initial level. An important example of this therapeutic procedure is provided by the intermittent treatment of leukemia L1210 with methotrexate.

c. Therapeutic regimens may be sought which will be most efficacious in delaying the origin of resistance in the tumor cell population.

d. Schedules of therapy may be sought which will be optimally effective in the treatment of tumor invasiveness, metastasis or sequested tumor cells. It is emphasized that once a drug has demonstrated antitumor activity it may be subjected to a series of experiments involving schedules of therapy which may improve therapeutic effectiveness.

2. Combinations of drugs

A number of examples are on hand in which, in the leukemia L1210 system and other systems, therapeutic synergism has been achieved by the use of combinations of drugs. A variety of agents have shown improved therapeutic effectiveness when employed in combination with methotrexate. Several examples include BCNU plus methotrexate, terephthalanilide 4',4"-bis-2-imidazolin-2-yl-amino plus methotrexate, methyl-GAG plus methotrexate, 5-fluorouracil plus methotrexate and methylnitrosourea plus methotrexate.

Therapeutic synergism has been observed not only with concomitant administration of drugs but also following sequential therapy. For example, sequential treatment with methotrexate plus 6-mercaptopurine provided an enhancement of therapy against advanced mouse leukemia L1210.

With combinations of drugs, as with individual drugs, the schedule of therapy may be critical for the achievement of maximum therapeutic effectiveness. An example of this is provided in studies of combination chemotherapy with azaserine and 6-mercaptopurine where therapeutic synergism was observed when the drugs were administered every two days in combination. The effect was diminished on daily administration and was not detectable when the drugs were administered twice daily.

Combination chemotherapy may involve not only the combination of antitumor agents but also the combination of chemotherapeutic agents plus protectants such as the combination of antimetabolites plus metabolites.

Detailed knowledge of the action of drugs with respect to cell cycle sensitivity and tumor cell kinetics may provide additional important applications of scheduling and combination chemotherapy.

C. A Model System for Testing the Antitumor Effectiveness of Combinations of Three Drugs. (A. Goldin, J. M. Venditti, N. Mantel, I. Kline and M. Gang)

Methodology for the investigation of the therapeutic efficacy of 3-way drug combinations was developed and illustrated using the experimental L1210 tumor system. The experimental design involves the testing of the drugs individually, in all possible pairs at a series of ratios, and in 3-drug combinations at a series of percentage compositions of the individual drugs. Any particular design is equivalent to the selection of a set of points at the vertices, along the edges, and in the interior of the equilateral triangle so that each point identifies the relative proportion in which the three drugs are to be used. Moreover, for each such relative proportion a range of dose levels is employed in order to bracket the optimal dose for that combination.

The drug combinations tested included (a) cytoxan, plus methotrexate plus TIC (NSC 60339, terephthalanilide, 2-chloro-4',4"-di-2-imidazolin-2-yl) and (b) cytoxan plus methotrexate plus bis-β-chloroethylnitrosourea (BCNU). Although in the present study there was no clear evidence of therapeutic synergism, the feasibility of evaluating 3-way drug combinations was demonstrated and the usefulness of the design triangle in such studies illustrated. The design triangle in essence permits ready visualization both of the experimental design involved and any patterns of synergism indicated by the resulting experimental data.

The increasing importance of combination chemotherapy in the treatment of neoplasia emphasizes the need for models which permit precise drug evaluation of combinations of drugs.

VI. Chemotherapy Studies

A. Chemotherapy of Metastases (S. R. Humphreys, K. Karrer, W. D. DeWys, and R. Johnson)

Additional studies have been conducted, using an experimental metastatic tumor system described in last year's report, which simulates the condition of patients with operable cancer plus metastases.

Lewis lung tumor implanted into the hindleg of BDF_1 mice grows as a primary tumor at the site of implantation, but the prognosis is determined by the metastases that develop primarily in the lungs early in the course of the disease. Because of drug toxicity complications in the presence of the primary tumor mass, chemotherapy alone was almost completely ineffective in increasing the survival of the mice with tumor. Radical surgical removal of the primary implanted tumor was only effective early in the course of the disease before pulmonary metastasis had developed. Chemotherapy as an adjuvant to surgical treatment was very effective under certain conditions.

When continuous and limited courses of cyclophosphamide (NSC 26271) were employed, the dose schedule, duration of treatment, and time relation between surgical treatment and the start of chemotherapy appeared to be of particular importance. Cyclophosphamide, administered every 5th day at dose levels of 225 or 168 mg/kg for a short course of 3 treatments, was more effective in increasing survival than continuous treatment.

Reports presented at the AACR and FASEB meetings showed that a few moderately large doses of cyclophosphamide given at 5 day intervals over a relatively short period of time were more effective than single dose treatment or continuous treatment in increasing survival of mice with metastases after surgery and that post-operative chemotherapy was more effective than pre-operative chemotherapy because of the establishment of new metastases as the result of the rapid recovery of the primary tumor cells after chemotherapy.

A report presented at the International Congress of Chemotherapy meeting in Vienna described the results of treatment with 16 chemotherapeutic drugs of current clinical interest and recently this number has been increased. Cyclophosphamide and the two urea derivatives BCNU (NSC 409962) urea, 1,3-bis(2-chloroethyl)-1-nitroso and (NSC 91741) urea, 1-(2-chloroethyl)-1-nitroso-3-(2,6-xylyl) were the most active in this system. Other alkylating agents and representative drugs from other classifications were ineffective. The importance of routes of drug administration and schedules of treatment in the selection of effective drugs was emphasized.

At a special meeting on the subject of cancer dissemination sponsored by the UICC in Milan, Italy, data was presented showing the effect of tumor and chemotherapy on the host, primary tumors and metastases. Following implantation of the tumor into the leg muscle the tumor grew progressively and metastasized to the lungs and later to the kidneys and other tissues. The course of the disease was marked by progressive anemia and leukocytosis. Chemotherapy with both cyclophosphamide and BCNU reduced the transplantability of tumor cells of the primary tumor and rendered metastatic cells incapable of surviving transplantation. But the primary tumor recovered rapidly and produced new metastases.

This experimental tumor system appears to respond to super voltage x-irradiation and chemotherapy in a manner similar to patients with Ewing's sarcoma and it is suggested that it may serve as a possible experimental model for the study of improved therapy of this disease. In several experiments neither x-irradiation nor chemotherapy of the primary tumor was effective in increasing survival of mice, but the combination of the two treatments produced a significant number of long-term survivors.

The experimental data from these studies suggest that chemotherapy as an adjuvant to radical surgical treatment of metastatic tumors can be extremely effective when properly employed and that well-designed animal experiments can be a useful tool in studying some of the general principles involved in the development of improved therapy. The following conclusions appear to be justifiable.

Chemotherapy as an adjuvant to surgical treatment appears to be more effective than surgical treatment or chemotherapy alone because:

1. Surgical treatment reduces the total number of tumor cells and eliminates the mass of cells which are the least vulnerable and perhaps least sensitive to chemotherapy.

2. Elimination, by surgical intervention, of the metabolic drain and physiologic disturbance of the primary tumor to the host permits the use of more effective dose levels of drug against metastasis without producing lethal toxicity to the host.

3. Extirpation of the primary tumor removes the site of residual tumor most likely to reseed metastasis after chemotherapy has completely eliminated metastatic tumor cells from the lungs.

4. Early metastases are poorly established but in very active uniform "log phase" tumor growth and may be particularly sensitive to chemotherapy in a manner similar to an early implanted tumor.

5. Cyclophosphamide appears to be a particularly effective chemotherapeutic agent against Lewis lung metastases since it may be activated primarily in the liver and circulated directly to the lungs. It may be that it is activated to some extent directly in the lungs. The metastatic tumor cells in the lungs may be particularly susceptible to alkylating agents or x-irradiation because of the high degree of oxygen tension in the lungs.

Short courses of chemotherapy are apparently more effective than continuous therapy because:

1. Dose levels of chemotherapy which have proved effective against metastases in one or several treatments are cumulatively toxic to the host when given continously.

2. Dose levels of drug which can be tolerated for continuous administration by the host are relatively ineffective against the metastatic tumor cells.

3. Low dose levels of drug which are relatively ineffective against tumor cells may eventually cause toxic effects as a result of tumor growth which also contributes to the general toxicity.

Experimental metastatic tumor systems such as employed in this study appear to be a more definitive means for evaluating new drugs for possible clinical use in patients with operable metastatic tumors than the implanted primary animal tumor systems now in general use.

B. Tumor Cell Synchrony in Relation to Chemotherapy (S. Vadlamudi)

Drugs such as amethopterin and cytosine arabinoside which exert their primary cytotoxic effect on DNA synthesis during the S phase of the mitotic cycle are limited in their therapeutic effectiveness by the heterogeneity of the leukemic cell population. It was considered that synchronization of the tumor cell population *in vivo* might permit more extensive exposure of the leukemic cells to the cytotoxic action of drug during the S phase.

Experiments were conducted to synchronize leukemia L1210 ascites cells employing the mitotic inhibitor colcemide. Colcemide arrested the leukemic cells in metaphase following which a significant degree of synchrony appeared to occur. Animals pretreated with colcemide were then treated with cytosine arabinoside at various subsequent intervals. The results of these studies indicate that colcemide alone was ineffective in increasing the survival time of the leukemic mice. Treatment with colcemide followed by treatment with ara-C resulted in therapeutic potentiation. A maximum increase in survival time occurred when ara-C was administered 15 hours after colcemide at which time (based on the studies of Skipper, et al) the cells were assumed to be in S phase. These studies are being extended to possible cell synchrony with vincristine, vinblastine, etc. The observations suggest the potential advantage of cell synchrony for the enhancement of therapy with antileukemic agents which exert their action during DNA synthesis.

C. Effect of Treatment with Cyclophosphamide on the Hematopoietic System of Mice. (W. D. DeWys)

Studies were conducted to follow the changes in the stem cell population (colony forming units), total marrow population and peripheral blood counts in mice following treatment with a large single dose of cytoxan. At twenty-four hours following treatment with cytoxan (275 mg/kg) the colony forming cells were reduced to 12% of the control values. This was followed by a period of exponential increase involving an over-shoot in colony forming cells by day 5 following which there was a progressive decline to normal values by day 9. The total marrow count showed a marked destructive phase that reached a nadir of 4% of normal by day 3. There then followed a period of exponential recovery and normal levels were reached by day 7 to 9. The decrease in peripheral blood counts following treatment with cyclophosphamide was, in general, parallel to the response of the bone marrow cells. However, the differential count did not return to normal until approximately 35 days following therapy. Preliminary investigations indicate that this information may provide a basis for the optimal timing of a second dose of cytoxan in terms of host tolerance to the drug. The suggestion is that drug tolerance may be greatest at the time of stem cell over-shoot. This question is under additional investigation. It is considered that elucidation of the cytokinetics of the hematopoietic system may permit more effective drug application in chemotherapy.

D. Resistance (W. D. DeWys)

Dose response studies in mice inoculated with a cytoxan resistant subline of leukemia L1210 indicated that the line was firmly resistant to small daily doses of the drug. However, larger intermittent doses of cyclophosphamide were capable of significantly damaging the tumor cell population. The studies suggest that the emergence of a clone of leukemic cells resistant to cytoxan would be favored by daily treatment with small doses of the drug. In contrast, intermittent treatment with larger doses of cytoxan might be capable of prevention of formation and of eradication of clones of resistant cells.

VII. Virus Leukemia

A. Rauscher Leukemia Virus. (S. Vadlamudi)

A study was conducted to determine whether terramycin might enhance the therapeutic effectiveness of streptonigrin in the treatment of Rauscher leukemia virus. Treatment with streptonigrin alone resulted in a 42% increase in lifespan of mice inoculated with Rauscher virus. Treatment with terramycin alone had little or no beneficial effect on the leukemic host. Treatment with a combination of streptonigrin and terramycin increased the lifespan of the leukemic mice more than 52%. Although the potentiation was not extensive it appears to be significant and the phenomenon is under further investigation.

B. Moloney Virus Lymphoma. (S. Vadlamudi, I. Kline and J. P. Glynn)

It had been demonstrated previously in this laboratory by Glynn et al that a series of lymphomas induced by the Moloney virus showed different degrees of sensitivity to chemotherapeutic agents. In additional studies the response of various lines of murine lymphomas induced by Moloney virus to treatment with cyclophosphamide was investigated. In agreement with the previous observations the results indicate that these tumors have different sensitivities to treatment with cyclophosphamide. In collaboration with Dr. George Klein of the Karolinska Institute determination will be made of the relative antigenicity of the lines having different sensitivity to drug. The suggestion is that those lines which are most antigenic may be the ones that are also most sensitive to chemotherapy.

VIII. Collaborative Studies

Collaborative studies with the Drug Evaluation Branch and the Biometry Branch, NCI were continued. (J. M. Venditti and staff, I. Kline, S. Vadlamudi and staff, W. D. DeWys, N. Mantel and A. Goldin)

In one study a system was devised for estimation of the duration of drug levels in mice as indicated by the residual antileukemic efficacy of compounds injected at various times prior to the time of inoculation of leukemic cells. With this system it was determined that cyclophosphamide had a half-life in the host of 17 minutes while 1-(2-chloroethyl)-3-

cyclohexyl-1-nitrosourea (CCNU; NSC 79037) had an estimated half-life in vivo of 94 minutes. Other related data are summarized in detail in the report of the Drug Evaluation Branch.

Collaborative studies were continued on schedules of drug administration, sequential and concomitant combination chemotherapy, etc. These studies are also presented in more detail in the report of the Drug Evaluation Branch.

A series of collaborative biological investigations was conducted to provide additional background information pertaining to the Lewis lung carcinoma. It was determined that C57Bl/6 and BDF_1 hybrid mice were most susceptible to the growth of this tumor, whereas BALB/c mice were most resistant to tumor implantation. Growth curves were established and the progressive increase in the number of tumor cells in the lung was followed. The doubling time of the tumor cell population was determined in relation to the growth curve. The details of these studies are summarized in the report of the Drug Evaluation Branch. The Lewis lung tumor appears to provide a suitable system for studying the influence of individual drugs and combinations of drugs and such studies are in progress.

Significance to Bio-Medical Research and the Program of the Institute

The chemotherapy research program is operated in direct relationship to the needs of the Office of the Scientific Director for Chemotherapy, NCI. The investigations are conducted as part of a collaborative program at the Kenwood Laboratories. The program is conducted in close coordination with the activities of the Drug Evaluation Branch, the Drug Development Branch, the Laboratory of Chemical Pharmacology and the Medicine Branch of the Cancer Institute. The research is dovetailed with the screening program and contract activities of CCNSC.

New methodology is transmitted to the screening program and pertinent basic observations are transmitted for possible clinical application. New drugs of clinical interest are investigated with respect to optimal usage and mechanism of action and recommendations are made to the clinic. Problems arising in the use of chemotherapeutic agents are subjected to additional investigation.

Proposed Course of the Project

The various aspects of the program will be continued and appropriate modifications made in accordance with the developments in the studies and the clinical interests.

Additional emphasis will be placed on detailed investigations with leukemia L1210, Lewis lung carcinoma and other naturally resistant tumors with a long generation time including characterization of drugs with respect to their action on the tumor cell mitotic cycle and tumor cell population dynamics. Additional tumor screening systems will be investigated with particular reference to drugs of clinical interest which are not active in the L1210 Leukemia-Walker carcinosarcoma screening combination. This includes tumor systems such as P388, P1534, the LPC1 plasma cell system, transplantable melanoma, viral-induced leukemias and functional and spontaneous tumors.

Detailed evaluation and investigation of drugs of potential clinical interest will be continued. Additional investigation will be conducted on scheduling procedures which will best permit recommendations for clinical use of new agents. New and novel scheduling methodology will be investigated. Studies will be continued to determine procedures for reducing drug toxicity and improving therapeutic effectiveness. This will include investigation of differential rates of recovery from drug toxicity of host tissues as compared with the tumor cell population. Studies with the spleen colony assay system will be continued as an ancillary index for the determination of optimal scheduling of therapy. Additional studies will be conducted on the maintenance of drug levels in the blood and transport, sites of action, etc. Biological and biochemical investigations will be continued to determine the characteristics and mechanism of action of antitumor agents. Additional studies will be conducted on the chemotherapeutic use of combinations of drugs and adjuvant chemotherapy. Additional attention will be focused on the phenomena of tumor invasiveness and resistance and potential chemotherapeutic approaches. Studies will be conducted on possible means for creating differential deficiencies of essential metabolite in tumor cells as opposed to vital cells of the host. An attempt will be made at purification of target enzymes. In collaboration with clinical investigators, biochemical investigations will be extended to human leukemic leukocytes and other tissues of interest. In relation to clinical interest, emphasis will be placed on studies of remission induction and remission maintenance. This will include study of suitable systems, characterization of drug action and factors which may influence the effectiveness of drugs in remission induction and remission maintenance.

Various facets of programs in progress and problems of particular clinical interest will be investigated.

Honors and Awards: Dr. Anthony W. Schrecker has spent 1967 as a Visiting Professor at Scripps Clinic and Research Foundation, La Jolla, California. Dr. Abraham Goldin and Stewart R. Humphreys participated in a Symposium on Methods to Study Cancer Dissemination and Metastases in Milan under the auspices of the International Union Against Cancer.

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MAMMALIAN GENETICS AND ANIMAL PRODUCTION SECTION
OFFICE OF ASSOCIATE SCIENTIFIC DIRECTOR AND CHIEF, CCNSC

Introduction

The Mammalian Genetics and Animal Production Section, during the year 1967, has been concerned with the provision of laboratory animals for the Chemotherapy Area, NCI. These services were also made available to various extramural program areas of the NCI, as requested. Changes in the Chemotherapy Program during this period suggested the activation of additional animal resources and the expansion of on-going projects. These "phase-ups" occasionally lagged slightly behind the increased requirements for rapidly changing programs. However, such happenings are not unusual for central services, and the ascents or descents to the various plateaus were attained without significant disruptions to studies in progress. These seeming operational complexities, however, were taken in stride and woven into the fabric of the overall program.

The Section's activities can be compared to a polyhedral-like configuration with two nuclei in its core. These nuclei, which are identified as (1) genetic characterization and (2) animal quality, work in partnership to furnish the cohesive force that is required to combine the constituent parts into a single entity. Its facets include axenic foundation colonies; the maintenance of animals with specifically defined microflora in flexible film isolators; colonies of animals in controlled environments; genetic-production centers which produce animals in conventional environments; large-scale animal production colonies; disease diagnostic laboratories; investigations concerned with the continuation of genetic characteristics for tumors and hosts; the effects of environment upon the microbial status of the animals; development of standards for the breeding, care, maintenance, transport and acquisition of laboratory animals; conferences in the areas of laboratory animal medicine and the application of laboratory animals in biomedical research; and the accreditation of commercial suppliers of laboratory animals for the testing and research programs of the NCI.

The Section staff furnish guidance and counseling for the development of animal colonies; provide assistance for the diagnosis and control of epizootics, technical information for facility design, information concerning the availability of animal stocks, equipment design and usage; and function as an information source for the biomedical community. Section staff members serve as members of national and area committees for the development of standards, organization of annual meetings of a scientific society, and as instructors in educational and training programs at the sub-professional and professional levels.

The animal production and supply program furnishes representatives of four species which include inbred, hybrids of inbred and non-inbred classes. These animals originate from prime centers maintained under closely controlled environments, and are used to stimulate the propagation of large populations in secondary centers. The latter, in turn, provide the bulk of the animals as breeders in large-scale production colonies and fewer numbers for selected investigations. In addition, these centers furnish pedigreed animals of various strains to laboratories in foreign countries which undertake investigations in collaboration with N.C.I. program areas.

Section staffing consists of one senior staff member, two staff assistants, one animal contracts assistant, and one clerk-stenographer.

The senior staff member and the assistants serve as Project Officer or Assistant Project Officer for the following contracts:

BDF_1 Hybrid Mouse Production	9
CDF_1 Hybrid Mouse Production	4
Inbred Mouse Production	3
Non-Inbred Rat Production	7
Non-Inbred Hamster Production	5
Provide Animal Husbandry Guidance and Pursue Selected Investigations	1
Inbred Rat Production	1
Beagle Hound Production	3
Genetic-Production Centers	6
Germfree Inbred Rodent Production	3
Germfree Inbred Rat Production	1
Germfree Non-Inbred Rat Production	1
Breeder Production Center for Hamsters	1
Disease Diagnosis and Research	3
Genetic Characterization of Tumors and Hosts	1
Standards for Animal Care and Maintenance	1
Conferences on Laboratory Animal Medicine and the Association of Animals in Biomedical Research	1
	51

The senior staff member also serves as Assistant Project Officer for animal husbandry guidance and counseling for research contracts.

Accomplishments

1. Animal Production

The Section continued to pursue its primary mission of providing genetically defined animals for a variety of testing and research programs. The requirement that the animals must demonstrate the desired histocompatibility characteristics controls the rate of acceleration of expanded production efforts. The input of breeding mice must be scheduled in a manner that will minimize peaks and valleys in the sine wave that represents a stabilized production level. Since the supply of animals must be closely correlated with weekly testing schedules, insufficient supplies create compound backlogs, and oversupply results in an economic wastage of effort and funds. Reduction in animal supplies can be accomplished in several ways. (1) If sufficient lead time is provided, colonies can be reduced by restricting the input of breeding animals over a period of two months, and (2) euthanasia can be accomplished in a matter of hours.

The reduction of a colony can be accomplished in less than 50% of the time required to restore production to the original level. Inbred and hybrid mouse production can be compared to an inverted pyramid, with the apex representing the foundation colony, the expansion colony located in a plane approximately 1/3 of the distance from the apex, and the base representing the production colony. If additional animals for testing are required, production in the expansion colony must be initially increased in order to produce additional breeding animals for the production colony. As a result, a minimum of three months is required to comply with demand. To once again borrow from geometry, the acceleration of production forms the hypotenuse of a right triangle whose vertical leg is the immediate increase in demand, and its horizontal leg represents the predicted plateau of compound testing. It would serve no useful purpose to immediately pump the full complement of breeders into a colony. This action would provide an overabundance of animals at a specific time, and nothing more during the pipeline period (gestation plus lactational periods) of approximately seven weeks. The foregoing factors are taken into consideration when plans are being developed for expanded production.

During 1967, the compound evaluation program employing the Walker 256 tumor system reached its peak level of activity. The base of six rat-producing colonies was considered as an adequate number of sources for additional rats. Subsequently, these colonies were able to achieve the elevated supply goals after additional breeding animals were introduced into the populations.

During the last quarters of 1967, the MG & APS was informed that the Chemotherapy staff was planning to de-emphasize the Walker 256 projects, expand L1210 testing, and possibly introduce several additional tumors within the spectrum of screening activities. In anticipation of these developments, the Section immediately embarked upon a program whose objective was to make available increased numbers of breeding animals from the various strains. By year's end, small groups of additional mice were being issued from the expansion colonies of the genetic-production centers. These were in addition to normal supplies.

Because of the accordion-like effect upon production levels imposed by Program changes, the Section found it necessary to expand the base of large-scale hybrid mouse production colonies. As anticipated, the output from these colonies did not exert a noticeable effect in reducing shortages until the end of 1967.

Once again, it was found necessary to increase CDF_1 hybrid mouse production in order to overcome shortages of BDF_1 hybrids for L1210 screening. The rate of acceleration of the latter hybrid was handicapped by the Section's inability to obtain sufficient C57BL/6 females from the Jackson Laboratory. This institution has served as the major source of DBA/2 males and C57BL/6 females that serve as the parents for the BDF_1 hybrid mice. During the past year, the production of the parent strains in genetic centers was increased in order to reduce dependence upon the Jackson Laboratories for these animals. The lack of sufficient animals at various times interfered with the orderly progress of the expansion phase of the production program. By year's end, however, the Section was producing sufficient DBA/2 males. Some progress has been accomplished in producing additional C57BL/6 females. Since four times the number of females in comparison to males are required, approximately six to nine months will be needed before the Section becomes self-sufficient for this strain.

Non-inbred rat supplies, procured by means of six production contracts, were adequate during the past year. The prime center which produced the breeding animals functioned smoothly. The techniques applied for this purpose represented a sophisticated approach to the creation of a colony with specifically defined microflora. The system served to furnish breeding animals free of pathogenic organisms. Samples of animals obtained from the production colonies revealed the absence of pathogens at this stage. The satisfactory outcome of these studies is self-evident. This three-stage system for animal production, which has been described in the past, constitutes a decided improvement over the methods that are normally applied for laboratory animal production.

Hamster usage increased during 1967 to a level of 2000 of these animals per week. It became necessary to activate three additional contracts for this purpose. Two of these resources supplied breeding animals from in-house colonies, and the third received Government-furnished breeders. The deadline for the delivery of weanlings obviated the provision of all breeders from the prime center. Nonetheless, the laboratory users reported that the health status of all hamsters was obviously better than it had been in previous years.

The prime center for hamster breeder production was again unsuccessful in developing a germfree or axenic colony of these animals. Although attempts by others have been equally unproductive, the Section does not console itself with this fact, and will continue its efforts to achieve this goal. The "clean-up" program, on the other hand, has been quite successful in establishing an isolator colony for the production of breeding animals. This center achieved its second stage goal of providing sufficient animals for two production colonies. Males in excess to this need were transferred to testing laboratories.

Beagle hounds were plentiful this year in contrast to the inadequate supplies available during 1966. This occurrence was not an unexpected development. An active sellers' market attracts investors in this field as well as other industries. Program requirements for these animals were approximately 50% of the need in 1966. Despite the abundance of beagle hounds, quality did not decrease. The prices remained stable despite the apprehension expressed by beagle producers that the terms of the Federal Animal Welfare Act, PL 89-544, would increase basic costs. These thoughts no doubt were applicable to sub-marginal producers, but the CCNSC accredited suppliers were exceeding the Act's standards prior to its implementation. All accredited suppliers received licenses from the U.S.D.A. without difficulty. Many were commended for the methods employed in husbandry practices and for their humane treatment of the animals. All beagles procured were applied satisfactorily in preclinical studies.

Large-scale production colonies furnished the following categories of animals:

Mice, Inbred: DBA/2, C57BL/6, and AKR/Lw.
Mice, Hybrid: BDF_1, CDF_1, and LAF_1.
Hamsters : Syrian (golden).
Rats : Fischer 344 and non-inbred albinos.
Dogs : Beagle hounds.

The total number of animals furnished to N.C.I. Program areas from all sources (Table I - appendix) represents a decrease of 71,850 during 1967 in comparison to 1966.

Program changes created a reduction in use rates for BALB/c, $CHKRF_1$ hybrids, and C57BL/6 mice, and Fischer 344 rats. A portion of the reduction was offset by a significant increase in non-inbred albino rats for the Walker 256 testing program.

2. Genetic Centers and Genetic-Production Centers

The conventionally-maintained genetic-production centers functioned at full capacity. Changes in laboratory program requirements for animals in turn suggested corresponding alterations in production levels. Major revisions to the original goals were due to the necessity of developing additional space for increases in DBA/2 and C57BL/6 production. In addition, several centers produced extremely limited numbers of certain strains and hybrids for developmental studies. The numbers of animals involved were insufficient for their production under the terms of fixed price contracts. In several instances, animals were provided for short term studies, and their propagation was performed without effort in the centers. The groups of animals included NZB, NZW and AKR/Lw inbreds; and $ZWZBF_1$, $D2AKF_1$, and CI^SF_1 hybrids.

Pedigreed litters and breeders for production colonies were shipped to Japan, India, Denmark and Israel. The offspring of these animals were used for chemotherapy studies performed under the terms of the U.S.-Japan Cooperative Science Program, the terms of a PL-480-funded contract with the Indian Cancer Research Centre Science Program, and collaborative studies with the CCNSC. All mice were vaccinated against ectromelia prior to shipment. The Section furnished informational publications, technical information by means of correspondence, and offered solutions by mail for problems when they occurred. The senior staff member has had the opportunity to meet with several representatives from the foreign laboratories when they visited this country. They reported that the resultant colonies were producing sufficient animals for their studies.

The conventional genetic-production centers maintained the following species and strains during 1967:

Inbred Mice: AKR/Lw, BALB/cAn, BALB/cJ, A/He, A/J, AL/N,
C3H/He, $C3H_f$/He, C57BL/6, C57BL/10Sc, DBA/2,

DBA/2Pl, CFW/Pl, NIH/Pl, PRI/Pl, CBA/J, SM/J, SJL/J, NZW, NZB, 129, and I^s.

Non-Inbred Mice: Non-inbred swiss which originated from Dr. C. Lynch's colony in 1937.

Hybrid Mice: $AKD2F_1$, CBF_1, CDF_1, CI^sF_1, and $D2AKF_1$.

Inbred Rats: F 344, OM/N, Wistar/Lewis, ACI 9935, Albany, S5B/Pl, M520 and NBR/Pl.

The germfree centers maintained by the A. R. Schmidt Company and the Charles River Breeding Laboratories advanced to the expansion colony stage during the past year. These colonies provided breeding mice for production colonies in controlled environments. Offspring were submitted to contract laboratories for evaluation and were found to be identical to conventionally reared counterparts for testing and research.

The germfree inbred rat colony at Tulane University School of Medicine successfully established germfree colonies of six inbred strains. Pedigreed offspring of three strains were transferred to secondary centers to initiate modified conventional colonies, and to laboratories for comparison studies. The contractor has been furnished several of Dr. W. Dunning's strains. When these colonies have reached sufficient size, pregnants will be selected for the subsequent processes involved for the establishment of axenic foundation colonies. This constitutes the first stage of the program to implement trust stocks of these valuable rat strains. The fact that such insurance colonies are not available at present has been a matter of concern to many people in the community. Once the various strains become established, pedigreed animals will be transferred to scientists (with Dr. Dunning's concurrence), who may express the desire to develop "in-house" colonies. We will provide guidance and counseling to these people for the continuation of the desired genetic characteristics.

During 1967, the following strains were established in the germfree centers:

Inbred Mice: DBA/2, C3H/He, C57BL/6, A/He, AKR/Lw, C57BL/10Sc, PRI/Pl, NIH/Pl, and 129.

Inbred Rats: Albany, ACI 9935, Buffalo, Fischer 344, Wistar/Lewis, OM/N, and NBR/Pl.

Clean Hamsters: Non-Inbred Syrian (golden).

3. Service Laboratories

The Battelle Memorial Institute (BMI) has continued to provide diagnostic services for the identification of Salmonella spp. and Pseudomonas spp. All positive Salmonellae of unusual serotypes were confirmed by the Ohio State Laboratories, and rare species by the NCDC at Atlanta.

Georgia. A single case of Salmonella typhimurium was detected, but it could not be reconfirmed by means of additional sampling. It may be that the sample was contaminated due to sloppy technique during the collection and packaging of the sample. During this period, the contractor supplied valuable assistance to an accredited supplier, although a non-contractor, who had developed a contamination in his colony. The most recent report indicates that the situation is under control. Overt salmonellosis was not demonstrated in this colony. Information supplied to accredited producers has assisted them in their efforts to reduce the incidence or eliminate P. aeruginosa from their animal colonies.

The Illinois Institute of Technology Research Institute (IITRI) has provided Salmonella and Pseudomonas diagnostic services similar to those furnished by BMI. In addition, IITRI operated a "trouble-shooting" laboratory for unusual conditions. The complete diagnosis program included virology, histopathology, bacteriology, and parasitology. Several studies were undertaken to identify differences in the microbial flora of several production populations, and the effect of certain microorganisms upon the animals' response to laboratory procedures. Preliminary evidence suggests that overgrowth of certain species influences the ability of mice to reach the required weight for testing programs.

The IITRI has undertaken a project to determine whether tumor growth characteristics differ when axenic and specifically defined microflora DBA/2 and CDF_1 are implanted with L1210. In addition, this laboratory is also studying compound sensitivities in these mice.

During 1967, the virus diagnostic laboratory services performed at the New Jersey College of Medicine and Dentistry became increasingly important. The profile of viruses has been expanded to include the following group:

Ectromelia, polyoma, LCM, PVM, Theiler's (GD VII), Sendai, Rheo 3, Echo, Toolan's H-1, SV5, MHV, MAV, and K.

Vaccine for the prevention of ectromelia epizootics has been supplied to all genetic centers, and to members of the biomedical community upon request. Several episodes which occurred during this period were quickly brought under control through the guidance and counseling supplied by Dr. Bernard A. Briody, the Principal Investigator. There is sufficient evidence that ectromelia is enzootic in the United States and, for this reason, colony managers and institutional personnel are encouraged to seek these services.

The inclusion of additional viruses rounds out our approach for the elimination of those which have been implicated in disease outbreaks in human as well as animal populations. Furthermore, it serves to identify, for purposes of elimination, the viruses which interfere with investigations in the areas of viral oncology and viral chemotherapy.

During this period, a project was undertaken to determine the presence or absence of LCM and other murine viruses in banked tumors as well as those used for studies in progress.

The histocompatibility studies performed at the Roswell Park Memorial Institute have been intensified during the past year. Reciprocal skin grafts involving pedigreed mice from the centers and the NIH and Jackson Laboratory colonies have been continued at nine month intervals. The reduction in interval was considered necessary in order to avoid missing a generation. All sublines, with the exception of one from the $C3H_f$/He strain, have been found to be histocompatible. The exception was discarded and replaced with a new start from the NIH. This laboratory has also, as required, investigated the iso-antigenic characteristics of tumors and the karyograms of aberrant tumor lines. The services provided by this contractor have enabled us to consider the entire program as a single package, and to furnish animals from any center to a specific laboratory without reservations.

The Mason Research Institute, under a contract sponsored by the Endocrinology Evaluation Branch, GL & C, has been performing skin grafts and iso-antigenic characterizations for inbred and non-inbred laboratory rats. The latter has not been pursued as vigorously for the rat in comparison to the multifold studies that have been followed for the inbred mouse. The data that is being accumulated will be of valuable assistance to users of inbred rats. This information will not only serve for selection of appropriate tumor hosts and/or those required for specific investigations, but will also assist investigators to evaluate the results of laboratory procedures.

The incidence of dead and ill animals on arrival during the year was 2.75%, an increase of 1.15% over the previous period. Much of this increase is directly related to undue exposure to extremely high or sub-freezing temperatures while in transit, since massive epizootics did not occur in production colonies during calendar year 1967.

4. The Accreditation Program

The Accreditation Program maintained by the CCNSC, Chemotherapy, NCI, was applied during the past year as basic criteria in evaluating the potential capabilities of animal producers as sources for the various species required for Program studies. During the past year, several provisionally accredited sources were dropped due to failure to improve facilities and methods. The Hartelust-Thorsen Company became the third accredited supplier of primates.

The MG & APS has observed that non-participants in the Accreditation Program continually improve their methods. Participants exceed in many respects the Accreditation Program Specifications, the I.L.A.R. Standards, and the guidelines suggested by the American Association for Accreditation of Laboratory Animal Colonies.

Section staff performed site visits to hitherto unaccredited responders to advertisements in the Commerce Business Daily. The organizations visited generally lacked appropriate facilities and/or competent staffing and/or experience in the laboratory animal production field.

Section staff were invited to visit commercial and institutional animal colonies for evaluation of methods and equipment. The advice of the Section was requested for improvements in facilities and facility design.

The Standards produced under Task Order No. 6 of Contract No. PH43-64-44 were included in contract specifications whenever they were applicable. During the past year, ad hoc committees were appointed to develop additional procurement standards, standards for the care and maintenance of axenic and gnotobiotic animals. The senior staff member of the MG & APS was appointed Chairman of the Committee on Nomenclature. The objectives of this committee are to develop uniformity in nomenclature for non-inbred laboratory animals, and to obtain the support of the editors of journals and authors to define the genetic and environmental background of animals used in research.

Task Order No. 16 of Contract No. PH43-64-44 arranged conferences on "Animal Models in Biomedical Research" and "The Laboratory Animal in Gerontology Research". "A Guide to Postdoctoral Training in Laboratory Animal Medicine" was published during this period.

During 1967, visits were made to animal colonies and laboratories as follows:

Germfree Genetic Centers for Rodents	21
Genetic-Production Centers for Rodents	27
Inbred Mice Production	4
Non-Inbred Rodents	120
Hybrid Mice Production	35
Contract Testing Laboratories	74
Service Laboratories	12
Miscellaneous Visits	82
	375

5. Technical Assistance

Request for advice and assistance were far in excess of those received during the previous period. Many were concerned with requests for recommendations to alleviate epizootics. The staff members continued to serve as members of on-going committees and were appointed to newly formed committees on the regional and national levels.

The Section presented its exhibit by request during the annual meetings of the American Veterinary Medical Association and the American Association for Laboratory Animal Science.

Proposed Course of Project

Plans have been developed to expand the basic colonies maintained in the centers in order to eliminate our reliance upon the Jackson Laboratory for breeding mice that are urgently required for the large-scale production colonies. Requirements projected for the Jackson Laboratory contract renewal period predict that 42% of the C57BL/6 females must originate from this source.

If the current rate of progress in the production of this strain in the centers continues, the CCNSC production program will become self-sustaining prior to the end of F.Y. 1969.

The controlled environment production colonies maintained by the germfree centers will be expanded within the limits of funds available. In any event, sufficient animals will be produced for evaluation studies. The germfree colonies which have become firmly established will serve as prime centers. During the 1968 calendar year, pedigreed litters will be shipped to conventional centers in order to reestablish foundation colonies.

Experience with filter covers during the past year has served to demonstrate their valuable contribution to the overall quality control program. Infant diarrhea has not been detected in the colonies, and these devices have materially assisted in the elimination or reduction of several agents such as polyoma and LCM.

The virus sampling program will be expanded to include commercial stocks on the premises of contractors. Assistance will be provided to the contractors in the event that certain viruses detected in their stocks may present hazards to contract colonies. All tumor lines in the frozen repositories will be tested for the presence of LCM and other viruses. The scientific community is being alerted to the fact that LCM in tumors and hosts presents a hazard to human health. This information has been broadcast by means of an informational publication.

The multiple source concept for large scale production colonies will be expanded in order to comply with the projected demand for BDF_1 hybrids during F.Y. 1969. Several promising sites have been identified and the managers' interest will be solicited.

As time and funds permit, the contractor at Tulane University School of Medicine will be encouraged to continue developing blood and other physiologic parameters for various strains of inbred rats.

The quality control program, in general, will be concerned with revisions in routine husbandry practices and the development of methodology which would serve to enhance the value of all animals used for the various investigations.

TABLE I

ANIMALS SUPPLIED DURING CALENDAR YEARS 1966 AND 1967

	1967	1966	+ Increase - Decrease
Hybrid Mice			
AKD2F$_1$	823	2,172	- 1,349
BDF$_1$	1,125,836	1,119,417	+ 6,419
CAF$_1$	--	913	- 913
CBF$_1$ (includes 8 litters) and 355 pregnants)	10,694	10,173	+ 521
CDF$_1$ (includes 8 litters)	191,125	219,295	- 28,170
CHKRF$_1$	--	23,646	- 23,646
CI^{s}F$_1$	699	--	+ 699
DBF$_1$	--	3,625	- 3,625
D2AKF$_1$	201	--	+ 201
LAF$_1$	1,190	5,087	- 3,897
ND2F$_1$	--	130	- 130
	1,330,568	1,384,458	- 53,890
Inbred Mice			
A/He	3,248	2,268	+ 980
A/J	2,082	1,809	+ 273
AKR/Lw	44,348	7,420	+ 36,928
AL/N	490	--	+ 490
BALB/cAn (includes 1118 pregnants and 266 litters)	297,139	405,900	- 108,761
BALB/cJ	537	31	+ 506
CBA/J	3,158	2,540	+ 618
CFW/Pl	1,753	2,164	- 411
C3H/He (includes 2255 pregs.)	35,824	30,299	+ 5,525
C3H$_f$/He	2,738	1,789	+ 949
C57BL/6 (includes 8 litters)	52,970	81,571	- 28,601
C57L	121	605	- 484
DBA/1J	45	25	+ 20
DBA/2 (includes 8 litters)	93,065	100,495	- 7,430
MA/J	23	--	+ 23
NIH/Pl (includes 177 pregs.)	2,869	1,130	+ 1,739
NZB	127	735	- 608
NZW	320	278	+ 42
PRI/Pl	1,921	1,708	+ 213
SJL/J	1,027	1,855	- 828
SM/J	883	965	- 82
129/J	--	400	- 400
	544,688	643,987	- 99,299

TABLE I
(Continued)

			+ Increase - Decrease
Inbred Rats			
Fischer 344	38,935	85,540	- 46,605
W/Lewis (includes 10 pregs.)	7,464	5,305	+ 2,159
Buffalo	4,077	1,977	+ 2,100
ACI 9935	3,890	2,361	+ 1,529
NBR/Pl (includes 6 pregs.)	1,434	49	+ 1,385
OM/N	37	28	+ 9
CA/S	45	12	+ 33
Albany	--	5	- 5
CA/R	--	24	- 24
	55,882	95,301	- 39,419
Swiss mice	31,366	116,855	- 85,489
Beagles	312	271	+ 41
Hamsters (includes 6 pregs.)	76,108	76,158	- 50
Non-Inbred Rats (includes 35 pregs.)	564,399	358,243	+ 206,156
Guinea Pigs	77	--	+ 77
Rabbits	14	--	+ 14
Monkeys	9	--	+ 9
GRAND TOTALS			
MICE	1,906,622	2,145,300	- 238,678
HAMSTERS	76,108	76,158	- 50
INBRED RATS	55,882	95,301	- 39,419
NON-INBRED RATS	564,399	358,243	+ 206,156
GUINEA PIGS	77	--	+ 77
RABBITS	14	--	+ 14
MONKEYS	9	--	+ 9
BEAGLES	312	271	+ 41

Publications:

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2. Cameron, T. P., Sevy, C., Carren, D., Cooper, L., McKeown, D.: An aid in the repair of the anterior cruciate ligament. J. of Veterinary Medicine/Small Animal Clinician. In press.
3. Poiley, S. M.: The development of an effective method for control of epizootic diarrhea in infant mice. J. Laboratory Animal Care 17: 501-510, Oct. 1967.
4. Poiley, S. M. : An improved method for limiting the introduction or transmission of pathogens in production and research animal areas. J. Laboratory Animal Care 17: 573-580, Dec. 1967.
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ANNUAL REPORT

DRUG DEVELOPMENT BRANCH

CHEMOTHERAPY
NATIONAL CANCER INSTITUTE

Introduction and Scope

The Drug Development Branch is engaged in a cooperative approach to the problems of cancer chemotherapy through the acquisition of chemicals and drugs for use in a broad program involving primary screening, secondary evaluation, toxicological studies, clinical trials, and the certification of the quality of those materials obtained for clinical and pharmacology studies. The primary responsibilities of the Drug Development Branch are:

1. The procurement of new compounds via chemical synthesis, contribution, and purchases for the primary screen;

2. The procurement of new antibiotic filtrates and extracts of plants and other natural products for the primary screen, and the isolation, purification, and identification of those materials that have shown activity in the screen;

3. The procurement and resynthesis of bulk chemicals and drugs, including plant and animal products and antibiotics, for toxicological evaluation and for clinical trial;

4. The procurement and synthesis of radiolabeled compounds for drug distribution and metabolic studies;

5. The chemical quality control of bulk chemicals and formulated drugs for use in clinical study or for preclinical toxicological and pharmacological evaluation;

6. The receiving, storage, and distribution of bulk chemicals;

7. The accessioning and chemical nomenclature of synthetic compounds, plant materials, and antibiotics entering the program;

8. The synthesis of new compounds for rapid exploration of leads developed in the screens;

9. The development of dose formulations and the procurement of clinical dose forms for clinical use; and

10. The storage and distribution of all formulated drugs for clinical use.

The methods for accessioning of new agents for in-put into the primary screen is gradually changing. More emphasis is being directed towards the selectivity of agents and less emphasis towards random procurement. Random sampling of the chemical universe cannot be abandoned completely because it continues to enjoy success in providing active materials for the program. However, as the structure-function mosaic gradually takes shape the choice of synthetic compounds for introduction into the screen will emerge more from direct synthesis and will gradually replace random screening. The gradual mutation of randomization to directed selection of agents for screening will be generated from review and evaluation of chemical and biological data from within the program, in the world of literature, and other related programs. Considerations are directed towards antitumor structure-activity relationships with emphasis towards new and promising structural classes and de-emphasis or elimination of adequately studied classes or non-productive structural classes.

New chemical structures or analogs developed through biochemical cell cycle and kinetics studies, pharmacologic and pharmaceutical rationales, and biological activities demonstrated in non-cancer programs should provide additional in-put to the directed synthesis approach. Chemical modification and structural analogs of natural products will also provide unique compounds of interest. The continued follow-up of empiric observations and changes in the structure of the already effective agents will also add to our array of new materials for investigations.

Directed synthesis will also be aimed at improving those agents which have shown therapeutic effectiveness in the screen but have been abandoned later because of toxicological or other problems. The random but unique structures acquired from natural products--higher plants, fermentation products, algae, marine and insect products--will continue to be explored.

With the increasing number of materials accessioned combined with the increasing number of active materials appearing with structural similarities, the Branch has brought together a group of top medicinal and organic chemists in an extensive review of the structure-activity relationships of agents in the Chemotherapy Program. The net result of such reviews will be to focus attention in synthetic areas of greatest potential and provide the chemist with more directed synthetic approaches and less random synthesis. This is not to be intrepreted that new structural classes will be by-passed, but that the effort will be concentrated in certain areas of chemistry providing fruitful leads. It is anticipated that additional comprehensive reviews in other areas will likewise provide drugs of paramount interest. A conference on the quinone moiety is being planned for the near future.

The Drug Development Branch manages a total of 38 contracts, totalling approximately six million dollars, covering 7 major areas as shown under the primary responsibilities of the Branch.

The Drug Development Branch has maintained a steady flow of materials into the program. These materials consist of chemicals, plant products, marine and insect products, and fermentation products. Some of these materials have demonstrated confirmed activity in one or more of the primary screens (Step III) and have been assigned as candidates for preclinical toxicological evaluation (Step IVA). An impressive number of Investigational New Drug Applications (IND) have been submitted to the Food and Drug Administration (see Table I).

In comparison with 1966, Table I reflects an increase in the number of crude extracts from all sources entering the program. A major increase in the number of crude extracts is noted in the plant and animal product category, with a modest increase in the fermentation products. In contrast to this increase, there has been a decrease in the number of synthetic materials entering the program. Since the greatest number of active materials in the past have come from the synthetic category, it is expected that as the in-put of synthetics into the program decreases the total number of active materials will decrease. The number of confirmed actives, Step III, and materials assigned for preclinical toxicological evaluation has remained essentially the same. A significant drop in the number of drugs filed for IND (Investigational New Drug) with the Food and Drug Administration is recorded in Tables I and III.

Of the 7413 new and crystalline materials added to the program in 1967, approximately 875 compounds (11.8%) were acquired under contract, 237 compounds (3.3%) were purchased, 2773 compounds (27.6%) were voluntarily submitted from industrial sources, and 3528 compounds (47.3%) were from non-industrial sources.

In spite of the decrease in the number of synthetic materials entering the screen in 1967, an increase of 2 confirmed actives (Step III), for a total of 180, were recorded. Eight synthetic materials were selected for preclinical toxicological evaluation, Step IVA, (Table II), and 5 IND applications, (Table III), were filed with FDA.

Of the 6253 plant products, approximately 58% from contractors, 166 provided crude active extracts. Sixty-seven crystalline materials were isolated from plant extracts and 12 have shown confirmed activity (Step III). Four materials of plant origin were selected for preclinical toxicological evaluation, (Table II), and one IND was filed, (Table III).

From the 4982 fermentation products, 50 provided crude active extracts. Forty-four were isolated in crystalline form and 8 have shown confirmed activity (Step III); 2 have been selected for preclinical toxicology (Step IVA), (Table II); and 1 IND was filed (Table III).

Of the 446 animal products, 18 crude extracts have shown activity. No crystalline materials have been isolated during this period.

From the 78 endocrine materials, 1 has confirmed activity (Step III); no new materials have been assigned for preclinical toxicological studies (Step IVA) (Table II); and 1 IND has been filed (Table III).

The interest in Natural Products has steadily increased as shown by the increase from 7904 in 1966 to 11,778 in 1967 in natural products extracts. The 2.5 fold increase in active crude extracts has been most gratifying. Effort will be accelerated in the coming months to fractionate and isolate the active component from these crude materials. Even though no active materials have been demonstrated from the algae or marine products, it is anticipated that these sources will provide interesting materials. It is of particular interest to note that the insect products are beginning to demonstrate a number of active agents.

Since a substantial number of compounds are contributed to the program on a voluntary basis, good public relations must be and have been maintained with the industrial firms and academic personnel by personal contact, by prompt reporting of data, and by making special handling of unusual agents a matter of normal service. With the increasing complexity of data processing and growth of the program, constant awareness of the problems of "commercial discreet" data must be ever present. Approximately 1543 individual suppliers have contributed samples to the program representing 887 industrial organizations, academic institutions and other government agencies. During 1967, there have been 139 new non-industrial suppliers and 14 new industrial suppliers submitting materials for primary screening. Even though the majority of these contributors are domestic suppliers, many materials have been supplied from foreign countries. The large number of contributors is an index of the enthusiasm for the program in the scientific community.

An increasing amount of effort is being directed toward procuring larger quantities of samples. Most organic synthesis conducted in the university laboratories is now done on a micro or semi-micro scale with publication as the major objective rather than the utility of the material. Coupled with this problem is the fact that fewer compounds are being generated via research grants than was experienced earlier. Therefore, samples from some university sources are no longer as readily available in adequate quantity as was true earlier. This problem, in all probability, will increase with time, and considerable added effort will be necessary to maintain the present rate of acquisition of compounds for routine testing. Combined with this problem is the fact that the number of duplicates being submitted is increasing. In contrast to this problem, a larger percentage of more exotic compounds of new types will enter the program.

The efforts in the area of development of new drug formulations and production of dose forms have accelerated significantly during the past year. A total of 33 development projects were assigned during 1967. Of the 33 development projects 10 new materials, not previously formulated, were developed in 1967. Of these, 5 were formulated for parenteral use and 5 were processed for oral administration. The production record accounts for 1,845,580 compressed tablets; 220,360 hard gelatine capsules; and 203,705 vials. An average inventory of 91 different compounds, 166 different formulations involving 270 dose forms by lot numbers has been maintained.

During 1967, a total of 281,655 drug items was shipped from inventory. In certain situations it is desirable to compare more than one drug or a drug and a placebo under the same conditions. This is done by "double-blind" studies which require that the materials used are provided in dose forms that cannot be differentiated from each other except by code. There are 23 such coded studies in the inventory.

Assigned to the Office of the Chief of the Drug Development Branch during most of fiscal year 1968 has been a special task group which functions as a liaison between the staff, field clinicians, and the Food and Drug Aministration. This group issues requests for procurement of drug formulations in accordance with approved protocols of studies of cooperative groups and independent investigators. They secure and maintain properly filed FDA Forms 1573 and curriculum vitae of all accredited investigators in accordance with FDA regulatory procedures. Duplicates of subject filing are also supplied to the FDA. At present the files of curriculum vitae and properly executed Forms 1573 number 862, of which 183 were recorded during 1967. There were 307 investigators removed from the investigators list; these were deleted because of various reasons--studies completed, withdrawal from study groups, or investigators were inactive in drug investigation. This group also collect data from the several program sources and properly petition the FDA for assignment of an IND number allowing clinical studies to be sponsored in accordance with regulatory procedures. The FDA is supplied with the clinical data on each drug at least once annually, and any unanticipated toxicities in human studies are reported to FDA and other field clinicians when observed. They notify the staff of all new drug filings with the FDA as well as discontinuance of IND numbers. The discontinuance of an IND may be due to (1) completion of study, (2) lack of response, (3) unanticipated toxicity, (4) formulation impossibilities, and (5) lack of clinical interest. Protocol files of the studies of all cooperative groups as well as those of independent investigators are also handled by this staff.

The maintenance of Kardex filing of all clinical drug formulations and issuance of request forms to the shipping room for distribution of drugs to accredited investigators for protocol studies by either cooperative groups or by independent investigators is a function of this group. During this report period 4543 such requests were issued. At present the Kardex file enumerates 357 drug inventories sub-divided as follows:

Open Inventories	188
Inventories for Special Protocol Studies	32
Endocrines for Breast Group	50
Drugs Stored at Burrough Brothers	49
Quarantined Drugs	30
Miscellaneous	8

Returned drug receipts are recorded and available for FDA inspection. Files on all drugs which have reached approved clinical evaluation are under constant surveillance. These files are available to staff and visiting investigators who may desire to obtain information for publication, research projects, or as directive for further study.

The Branch, upon request, has reviewed and commented upon 122 Grant Proposals. The opinions expressed have been used extensively by the Grant Study Sections on their evaluation of the proposals. Such comments may include the number of materials submitted for testing, indicating those compounds showing promise as potential antitumor agents.

Also upon request, the Branch has reviewed 3 patent applications. The applications are reviewed as to novelty, expected utility, and need to protect the public's interest by patent. The opinions and recommendations are used by the NIH Patent Section in their consideration of the application.

Staff

The Drug Development Branch is composed of a staff of 27 persons, consisting of five senior professional scientists (GS-13 and above), eight other professional staff members (GS-9 and above), three PHS Commissioned Corps Officers, and the remainder technical and clerical staff.

The Branch is divided into four Sections. Those responsible for each activity are as follows:

Drug Development Branch
Chief - Dr. Harry B. Wood, Jr.

Chemical and Drug Procurement Section
Head - Dr. Robert R. Engle

Chemical and Drug Records Section
Head - Mr. Robert B. Ing

Clinical Drug Distribution Section
Head - Mr. J. Paul Davignon

Natural Products Section
Head - Dr. Jonathan L. Hartwell

The Chemical Quality Control Unit (Head, Mr. C. A. Hewitt) operates as an activity under the Chemical and Drug Procurement Section. The Nomenclature Unit (Head, Mrs. M. Flather) functions under the Chemical and Drug Records Section. The Antibiotic Unit (Head, Dr. A. Stanley) operates as an activity under the Natural Products Section. A Special Task Group for liaison with FDA (Head, Dr. A. Osterberg) is assigned to the Office of the Chief.

Accomplishments

In summary, the following were achieved during calendar year 1967 by the Drug Development Branch:

1. A total of 18,983 new substances (Table I) were obtained for screening or other evaluation;
    - a) 7224 synthetics
    - b) 4982 antibiotic cultures
    - c) 6253 plant materials
    - d) 446 animal materials
    - e) 78 steroids.
2. Of the above compounds, 921 were prepared under contract;
    - a) 108 from the preparation laboratories contracts
    - b) 790 from the synthetic laboratories contracts
    - c) 23 from endocrine contracts (under General Laboratories and Clinics).
3. One-hundred and seventy-six compounds were resynthesized in the preparation laboratories.
4. Fourteen new substances were assigned to Step IVA (Table II) (Candidates for preclinical toxicology);
    - a) 2 fermentation products
    - b) 4 plant products
    - c) 8 synthetic compounds.
5. Eight drugs were assigned IND numbers (Table III);
    - a) 1 endocrine compound
    - b) 1 fermentation product
    - c) 1 plant product
    - d) 5 synthetic compounds.
6. Forty-one IND drugs were withdrawn.
7. Two-hundred and thirteen compounds were assigned to Step IVB.
8. Two-hundred and one compounds were assigned to Step III (Table I).
9. Analytical-quality control determinations were made on 173 different lots of 84 different compounds.
10. Twelve radioactive labeled compounds (Table IV) were procured.
11. Twenty-nine dose formulations were developed.
12. Ten new materials were formulated of which eight were filed with FDA during 1967.

13. Approximately 2,269,645 formulated items were prepared;

    a) 1,845,580 compressed tablets
    b) 220,360 hard gelatin capsules
    c) 203,705 vials.

14. Thirty-eight contracts were supervised by the Branch;

    a) 7 preparation laboratories
    b) 6 new synthetics
    c) 13 plant products
    d) 5 fermentation products
    e) 2 analytical quality control
    f) 3 drug formulations
    g) 2 miscellaneous.

15. There were 122 Grant Proposals and 3 Patent Applications reviewed and commented upon.

16. Samples were received from 14 new Industrial concerns.

17. Materials were received from 153 new sources.

18. Sixty-two manuscripts by contractors were cleared for publication.

TABLE I

MATERIALS ACCESSIONED AND ACTIVITY REPORT(1)

	CRUDE EXTRACTS				CRYSTALLINE							
	NO. SUBMITTED		NO. ACTIVE		MATERIAL SUBMITTED		STEP III(2)		STEP IVA(3)		IND FILED	
	1966	1967	1966	1967	1966	1967	1966	1967	1966	1967	1966	1967
Fermentation Products	4284	4982	72	50	32	44	1	8	2	2	--	1
Animal Products	88	446	27	18	--	--	--	--	--	--	--	--
Plant Products	3532	6253	89	166(4)	57	67	21	12	6	4	--	1
Endocrine	--	--	--	--	171	78	--	1	1	--	10	1
Synthetics	--	--	--	--	7577	7224	178	180	9	8	9	5
Total	7904	11,778	188	234	7837	7413	200	201	18	14	19	8

(1) Based on calendar year.

(2) Showing confirmed activity

(3) Selected as candidates for preclinical toxicological evaluation and clinical trial

(4) Individual species

TABLE II

COMPOUNDS ASSIGNED TO STEP IVA DURING CALENDAR YEAR 1967

NSC NO.	COMPOUND NAME	SOURCE
71261	9*H*-Purine-6-thiol, 2-amino-9-(2-deoxy-β-*D*-*erythro*-pentofuranosyl)-, monohydrate	Synthetic
71795	6*H*-Pyrido[4,3-*b*]carbazole, 5,11-dimethyl- Ellipticine	Natural Product
71851	9*H*-Purine-6-thiol, 2-amino-9-(2-deoxy-α-*D*-*erythro*-pentofuranosyl)-	Synthetic
80087	*p*-Toluidine, α-inden-1-ylidene-*N*,*N*-dimethyl-	Synthetic
80439	4,7,10,13-Tetraoxahexadecane, 1,2:15,16-diepoxy-	Synthetic
93150	Cytosine, 1-β-*D*-arabinofuranosyl-, 2',3',5'-triacetate	Synthetic
104800	Galactitol, 1,6-dibromo-1,6-dideoxy-	Synthetic
107392	Picolinaldehyde, 5-hydroxy-, thiosemicarbazone	Synthetic
108836	1*H*-Pyrazolo[3,4-*d*]pyrimidin-4-ol, sodium deriv.	Synthetic
109229	L-Asparaginase	Fermentation
110372E	β-Sitosterol hydrogen[(carboxymethyl)thio]succinate	Natural Product
110435	Protein, unknown	Natural Product
113233	Active Principle from B-2992	Fermentation
403169	Acronycine 7*H*-Pyrano[2,3-*c*]acridin-7-one, 3,12-dihydro-6-methoxy-3,3,12-trimethyl-	Natural Product

TABLE III

DRUGS FILED WITH FDA IN 1967

NSC NO.	DRUG	IND NO.
11905	Lapachol	3846
85998	Streptazotocin	3859
75520	Uridine, 2'-deoxy-5-(trifluoromethyl)-	3953
26271	Cytoxan**	4173
88536 E	Androst-4-en-3-one, 17β-hydroxy-7β,17-dimethyl-	4401
	(Testosterone, 7β,17-dimethyl)*	
3590	**Citrovorum factor**	4292
740	Methotrexate**	4291
82196	Imidazole-4(or5)-carboxamide, 5(or 4)-[3,3-bis(2-chloroethyl)-1-triazeno]-	4338

*Other names shown in parenthesis

**New formulations of commercial drugs

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CHEMICAL AND DRUG PROCUREMENT SECTION

DRUG DEVELOPMENT BRANCH

Scope

The function of the Chemical and Drug Procurement Section is to acquire synthetic compounds for antitumor evaluation, to procure large quantities for advanced screening, confirmational testing, toxicological and pharmacological evaluation, and clinical trial in humans, and to procure radiolabeled materials needed for pharmacological and clinical studies. The Section plays a significant role in the activities of the entire Chemotherapy Program, as well as that of the Branch.

The work of the Section is divided into three principal areas:

1. The Preparative Activities, which synthesize
    a) Bulk chemicals and drugs
    b) Radiolabeled materials
    for toxicological, pharmacological, and clinical investigations, for confirmational testing, and for completion of primary evaluation;

2. The Synthesis Activities, which provide new compounds for primary screening; and

3. Analytical-Quality Control Activities, which provide the analytical check on the identity, assay, and purity of compounds and formulated drugs entering the pharmacological and clinical programs. Solubility and stability data are also acquired as is structural analysis.

Staff

The staff of the Chemical and Drug Procurement Section is presently composed of three professional people and one secretary. One professional person is involved full time with the quality control activities of the Section. The professional staff members serve as project officers on the various contract activities of the Section as well as on other contracts that are monitored by the Drug Development Branch.

Preparative Activities

The resynthesis or preparation laboratories are, in the strictest sense, service laboratories, designed and selected to prepare known chemicals and bulk drugs which are needed by the program, yet are not available on the open market or from the original suppliers. They differ from the synthesis contracts in that the synthesis contracts are devoted to preparing entirely new compounds with diverse chemical structures.

Seven preparation laboratories are directed by the Section. These laboratories were selected on the basis of ability of the principal investigator, the facilities available, the capability of the company in the field of chemistry related to the Cancer Chemotherapy Program, and the adaptability to synthesis of new types of compounds.

TABLE IV

CONTRACT PREPARATIONS LABORATORIES FOR CLINICAL AND EXPERIMENTAL COMPOUNDS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Collaborative Research, Inc.	Friedman	PH43-66-506
Merck and Co.	Babson	PH43-62-479
Monsanto Research Corporation	Yanko	PH43-65-1044
Monsanto Research Corporation	Garner	PH43-67-658
Parke, Davis and Company	Dice	SA-43-ph-4320
Riker Laboratories	Keller	PH43-66-904
Starks Associates, Inc.	Starks	PH43-62-484

The preparation laboratories are also used to obtain cost data for the preparation of the necessary quantities of clinically-important chemicals and to develop the most economical means for their preparation. Usually a method and/or reference for the synthesis of the compounds requested are supplied to the contractors by this Section. It should be pointed out that many methods of synthesis which are practical for a small quantity of chemical are not technically feasible or economically practical when used for a large-scale synthetic operation or for radiolabel synthesis. The conversion of small-scale to large-scale production often requires developmental studies which are conveniently carried out by the preparation laboratories. Solubility and stability studies have also been performed by the preparation laboratories upon request. In addition, some of these contractors have provided material in formulated dose form.

a) Bulk chemicals and drugs

The compounds chosen for resynthesis at the preparative laboratories are not available in the quantities needed from the original supplier or on the open market. The materials are assigned for resynthesis for five major reasons:

1. For clinical investigations;
2. For preclinical toxicological and pharmacological evaluation;
3. For maintaining a stock of chemicals of clinical and preclinical interest for study in the program;
4. For completion of screening or confirmational testing; and
5. For use as intermediates in further synthesis.

The compounds so chosen are placed on priority lists by this Section based upon their relative importance, with the toxicological and clinical compounds given top rating. The quantity of a given material to be resynthesized may vary from 1 gram to occasionally as much as 75 kilograms. Factors governing the amounts depend upon such considerations as: use, ease of preparation, stability, and cost. Materials of high quality are prepared in the preparation laboratories. Every effort is made to safeguard the clinician in the quality of the drug he receives.

The preparation laboratories, taken collectively, provide the means of obtaining nearly any type of chemical compound, regardless of structure, and the ability of providing large quantities of very high purity drugs which may be needed for the program.

The Chemical and Drug Procurement Section, through the preparative laboratories, directed the resynthesis of 279 compounds totaling 42.2 kilograms during the calendar year 1967. Included in this group are 19 compounds scheduled for or undergoing clinical trial totaling 36.9 kilograms. The Section procured from all sources including purchases a total of 32 compounds scheduled for or presently in clinical trial amounting to 166.8 kilograms of material.

b) Radiolabeled materials

Due to the increased emphasis being placed on pharmacological and drug distribution studies, the need for radiolabeled chemicals and drugs has greatly increased. These agents are valuable tools in such studies and the procurement of them is coordinated by this Section.

One contract (PH43-65-1044) is devoted to the procurement of radiolabeled materials for use in preclinical pharmacological and clinical studies. If available, the required labeled materials are purchased from commercial sources. Materials not available from commercial sources and needed by the Program are prepared by this preparative laboratory. This contract also handles the storage and distribution, and performs the necessary analytical work for all labeled materials, whether prepared under the contract or acquired from other sources. All requests for radio-active materials are reviewed by a committee in relation to proposed use prior to assignment to the contract for preparation. During this report period, the Section procured from all sources a total of 12 radio-labeled materials (See Table V) and directed their distribution. Thirty shipments of radio-active substances were made during calendar year 1967.

TABLE V

RADIOLABELED MATERIALS

NSC NO.	COMPOUND	LABEL
740	Methotrexate	$3',5'-H^3$
755	Purine, 6-thiol-	S^{35}
755	Purine, 6-thiol-	$8-C^{14}$
3073	Folic acid	$3',5'-H^3$
29630	3',5'-Dichloromethotrexate	$3',5'-Cl^{36}$
38297	Uridine, 5-bromo-2'-deoxy-	$6-H^3$
45388	Imidazole-4(or 5)-carboxamide, 5(or 4)-(3,3-dimethyl-1-triazeno)-	C^{14}(methyl)
45388	Imidazole-4(or 5)-carboxamide, 5(or 4)-(3,3-dimethyl-1-triazeno)-	$2-C^{14}$
58404	Glycine, N-diazoacetyl-, hydrazide	$1-C^{14}$
79037	Urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-	H^3(cyclohexyl)
82196	Imidazole-4(or 5)-carboxamide, 5(or 4)-[3,3-bis(2-chloroethyl)-1-triazeno]-	$2-C^{14}$
	Sodium Radiophosphate P^{32} for injection	P^{32}

Synthesis Activities

The objective of the synthesis laboratories is to provide the program with new chemical agents for anticancer evaluations. The laboratories are designed to supply compounds of diverse structures and complexity. The compounds selected for preparation are based upon the following criteria:

1. Modification of clinically active compounds;
2. Leads generating from the screening program;
3. Biological and biochemical rationale;
4. Leads from the literature;
5. Compounds to explore structure-activity relationships;
6. New structural types; and
7. Agents to resolve questions on chemical and biochemical mechanism and mode of action.

The Chemical and Drug Procurement Section directs six contracts for the chemical synthesis of new compounds for the program.

TABLE VI

CONTRACT SYNTHESIS LABORATORIES

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Arthur D. Little, Inc.	Papanastassiou	PH43-65-502
Midwest Research Institute	Cheng	PH43-65-94
Chas. Pfizer and Co.	Oleson	PH43-68-45
Southern Research Institute	Montgomery	PH43-64-51
Stanford Research Institute	Goodman	PH43-64-500
University of Utah	Robins	PH43-65-1041

During the past year a review by a group of expert chemical advisors on the major areas of the synthesis program was conducted. The area of nucleosides was reviewed during this report period and the results of this review are reported in the discussion of the nucleoside effort below. Only two major areas have been reviewed to date--folic acid antagonists (which was reported last year) and nucleosides. Additional comprehensive reviews of other areas are planned for the future.

The efforts of the synthetic program are channeled into many areas of investigation; for example,

(1) A significant effort of the Section is devoted to the synthesis of analogs and derivatives of the naturally-occurring nucleosides. The study of nucleoside metabolism may be expected to contribute to the design of chemotherapeutic agents with antiviral activity against tumor-inducing viruses and may help elucidate many cellular processess in the synthesis of DNA and therefore of cell division. Nucleoside antimetabolites may

function by inhibiting the incorporation of normal constituents in RNA or DNA and preventing the formation of enzymes necessary for biosynthesis of the tumor nucleic acids. Also, the conversion of nucleosides to nucleotides may be structurally less restrictive than the conversion of the free base to the corresponding nucleotides.

In addition, the area of nucleosides has already provided a number of very interesting compounds capable of suppressing tumor growth in experimental animals and producing favorable clinical response in certain types of human cancer. Cytosine arabinoside, 5-FUDR, and 6-methylthiopurine riboside are nucleosides which have demonstrated clinical activity. However, further chemical studies are needed to provide nucleosides of greater specificity and less toxicity and which are resistant to enzymatic degradation in the cancer patient.

A wide variety of structural modifications and derivatives of nucleosides has been prepared and submitted for evaluation during this report period. These include (a) analogs of the nucleoside antibiotics such as tubercidin, toyocamycin, sangivamycin, formycin and formycin B, (b) a variety of pyranosyl nucleosides, (c) a number of 8-substituted purine nucleosides, (d) mercapto and amino sugar derivatives, (e) α-nucleosides, (f) 7-D-ribofuranosylpurine nucleosides, (g) 2-fluoro and 2,6-difluoro derivatives, (h) 3'-keto derivative, (i) 5'-carboxylic and analogs, (j) 3'-O-methyl derivatives, (k) imidazole and benzimidazole nucleosides, (l) branched chain sugar analog, (m) cyclopentane analog, (n) 1-substituted derivatives, (o) deazapurine nucleosides, and (p) nucleosides with natural bases with fraudulent sugars, with natural sugars and fraudulent bases, and with fraudulent bases and sugars.

Several interesting substances have been prepared in the area of nucleosides and a comprehensive review of this area was made during this report period by the group of expert chemical advisors and other experts in this area.

The purpose of the meeting was to guide the Chemotherapy Program in the design of new nucleosides, or in the more effective use of the existing ones. The review involved all phases of nucleoside activity from basic chemical work to clinical studies. A study of the structure-activity relationship of all the nucleosides which have been in the program was made in an endeavor to define the areas for further synthetic efforts in this field of chemistry.

The following conclusions resulted from the nucleoside review meeting:

1. The nucleoside area has been very fruitful and several compounds of clinical interest belong to this class of compounds. Also, there are some materials of this type which are showing interesting activity in preclinical evaluation.

2. The present synthetic program in the area of nucleoside chemistry is a reasonable approach for the funding available. The program encompasses variation in the base portion, sugar portion, or both.

3. A review of the screening data should be made to determine on which compounds additional biological data is needed and those select compounds acquired for completion of biological study.

4. There is an abundance of work yet to be done in the area of nucleoside chemistry. Some areas need to be investigated further while in others the investigations have yet to be initiated.

5. New and better methods for the preparation of compounds of this type are needed.

(2) A considerable effort of the synthesis program has been devoted to the preparation of analogs and derivatives of folic acid. Resistance to folic acid antagonists in L-1210 leukemia is frequently associated with a marked increase in the enzyme dihydrofolate reductase. Since this change is limited to tumor cells, the provision of a fraudulent substrate to the tumor cell would lead to the production of a fraudulent coenzyme thus producing a lethal synthesis in the tumor cell. Homofolic acid was prepared as a potential fraudulent substrate for dihydrofolate reductase in resistant tumor cells. Tetrahydrohomofolic acid, the end product of the reduction of homofolic acid by dihydrofolate reductase, has also been prepared and is a striking inhibitor of thymidylate synthetase. Tetrahydrohomofolic acid is presently being obtained in quantities sufficient for preclinical evaluation.

In addition, Methotrexate is an effective clinical agent and this molecule is considered to be worthy of further modification.

New folic acid analogs and derivatives have been prepared and the investigation in this area is continuing at an accelerated rate as a result of the comprehensive review of the area which was made in January, 1967, the results of which are reported this year. All present work is along the lines discussed and encouraged at the review meeting. The structure of the folic acid molecule has been divided into five parts and modifications have been undertaken in each area. The areas and examples of investigations are (a) ring A (pyrimidine) portion of the pteridine ring; 1- and 3- deaza analogs of folic acid, aminopterin, and Methotrexate; (b) ring B of the pteridine ring; 5- and 8-deaza and 7-aza analogs of the three basic structures; (c) the bridge; homo analogs of Methotrexate and aminopterin, 9,10-unsaturated (anils) derivatives; (d) the benzene ring; substituted heterocyclic and alicyclic rings; alkyl, alkenyl, and alkynyl chains; and halogens and other functional groups on the ring, especially in the 3'- and 5'-positions; and (e) the amino acid moiety; d-amino acids and basic amino acids.

Many times methods for the preparation of the antifolates must be developed using "model" compounds. Methods have been developed which provide routes to the 1-, 3-, and 5-deaza analogs of folic acid. Also, an improved method for the preparation of 6-substituted 2,4-diaminopteridines has been developed which should be applicable to the synthesis of homoamethopterin and homoaminopterin. In addition, the route to the preparation of analogs where the benzene ring is replaced by a heterocyclic ring has been developed during this report period.

(3) Some of the synthetic effort of the Section has been directed toward the synthesis of natural products, their analogs and derivatives. Several products are not available in the quantities necessary for complete evaluation from natural sources, and if the structure is known, attempts are made to prepare the material synthetically. Also the recollection of the plant materials is in many instances a major problem. Many times the investigation of a synthetic route to the preparation of a natural product affords, or affords the means to, the preparation of analogs which may be as active and interesting or more so than the natural material. It may also be desirable to prepare synthetic analogs and derivatives of naturally occurring material to develop the parameters of an interesting lead or in the hopes of reducing adverse toxicity while retaining activity. Recognizing that natural materials afford many interesting structures and compounds, quite often analogs and derivatives of these can be procured only by synthetic means.

Several of the clinically active compounds, such as Daunomycin, Streptonigrin and Actinomycin D, and materials which have demonostrated interesting antitumor activity, such as Lapachol, have a quinone moiety present. This Section has therefore initiated work in the synthesis of quinone type molecules. The synthesis of some compounds of this type was achieved during this report period and the work is being continued.

Another structural moiety which is present in several naturally occurring materials which are showing interesting in vivo activity is the α,β-unsaturated lactone or ketone. Synthesis of compounds containing this moiety has also been undertaken.

Since the clinically interesting alkaloids vinblastine and vincristine possess both a dihydroindole and an indole moiety, the Section has initiated a synthetic program in the indole alkaloid area. The simplified analogs of several polynuclear indoles are being prepared. Also, the preparation of some synthetic alkaloids containing the tryptamine unit, the condensed indole ring system, and the 3-azaindole (benzimidazole) ring system is being investigated.

In addition to the above-mentioned work based on the quinone moiety of Daunomycin, other synthetic analogs and derivatives of this clinically active antibiotic are being prepared. Analogs of both the aglycone, daunomycinone, and the sugar, daunosamine, as well as of Daunomycin itself are being prepared. The synthesis of daunosamine and other 3-amino-2,3,6-trideoxyhexoses has been achieved. A "model" synthetic aglycone has been prepared and methods for the attachment of the sugar portion have been developed. The sugar, daunosamine, as well as other sugars, both naturally occurring and amino sugars, have been attached to this model aglycone. Attempts are being made to attach other sugars to the natural aglycone, daunomycinone. Work in this area is continuing.

In addition to the above mentioned work based on the quinone moiety of the clinically active antibiotic Streptonigrin, other analogs and derivatives of this substance are being prepared.

The main synthetic effort relating to Streptonigrin-like compounds is directed towards the preparation of derivatives of rings A and B, rings C and D, and a systematic modification of the substituents of the parent substance.

An accelerated effort has been made during this report period towards the synthesis of the naturally occurring alkaloid, camptothecin. Since the quantity of this substance which can be obtained from natural sources is very limited and the extraction of it from the plant material is very costly, the total synthesis of this compound became of prime interest. Unfortunately, the synthesis of camptothecin has not been achieved to date but the work is progressing quite satisfactorily and it is being continued. Upon completion of the synthesis some analogs and derivatives will also be prepared.

Work on the preparation of derivatives and analogs of ellipticine is being continued. Also a practical synthesis of the parent compound, ellticine, has been developed and this material is being prepared in large quantities synthetically for preclinical evaluation.

(4) Some of the effort of the Section has been directed towards the synthesis of potential antimetabolites. One such structural type is the imidazole. Two imidazole-containing compounds have demonstrated activity against L-1210 leukemia and are undergoing clinical evaluation. In addition, compounds of this type have been shown to have broad spectrum antifungal and antibacterial activity. Thus, additional imidazole analogs and derivatives have been prepared and are being synthesized for evaluation as antitumor agents.

(5) The class of compounds known as N-nitrosureas are active against leukemia and some possess the ability to cross the blood-brain barrier. Of special interest are the results from intracerebrally-inoculated L-1210 and the use of maximal single dose (one day only) treatment schedules. The testing in rodents with some compounds of this type has resulted in a significant number of cures based on thirty-day survivals. Additional examples of this structural type have been synthesized and submitted for antitumor evaluation.

(6) Only a limited amount of effort is being devoted to the synthesis of alkylating agents other than those of the N-nitrosurea-type since several agents of this type have been clinically evaluated. A small amount of effort is being devoted to the preparation of analogs and derivatives of Cytoxan. This work is limited to the identification and preparation of the therapeutic metabolite and the hydrolytic products of Cytoxan.

(7) In view of the antileukemic and antiviral activity of thiosemicarbazones and guanylhydrazones, compounds of these classes have been under investigation. The thiosemicarbazone of 5-hydroxypicolinaldehyde is undergoing preclinical toxicological studies. Additional derivatives and analogs of this type are being prepared for further evaluation in this area of chemistry.

(8) A small effort was devoted to the study of a new class of compounds to the Program, namely, the lower molecular weight histones. Histones are basic proteins (or polypeptides) found in the nuclei of the cells. Recent reports have suggested that polycationic compounds in cell nuclei repress the active chromatin and/or act as inhibitors in the RNA biosynthesis. A number of such macromolecular biodegradable polycationic compounds which may alter the synthesis of the cell nucleus by combining with DNA and/or acidic proteins and disrupt the metabolic process in the cell were prepared and submitted.

One very important advantage of synthesis contracts of this type is that they are placed with laboratories which are able to offer a quick response to a new lead and move quickly into an area of research generated by the Chemotherapy Program and the various clinical groups. The broad experience and mature judgment in synthetic chemistry of these laboratories place them in a unique position and have been of great value to the program. These contractors have provided a great number of compounds with a wide variation in chemical structure. The principal investigator under each contractor is extremely competent and well-versed in the areas of chemistry under investigation. Each laboratory is well-organized. The physical facilities in each case are modern, well-equipped, and supported by very progressive managements. All of these contractors are definite assets to the Chemotherapy Program.

During the past year, fourteen new compounds were selected for Step IVA, of which eight were synthetic materials. The new synthetic compounds which were assigned to Step IVA are listed on Table II.

QUALITY CONTROL

The Branch is keenly aware of the need for providing bulk chemicals and formulated drugs of highest quality for use in clinical investigations and preclinical evaluations. The purpose of quality control, based on rigorous chemical analytical methods, is to evaluate these materials as to their identity, assay, and purity. All lots of bulk materials to be used in preclinical toxicological and pharmacology or in clinical studies are sent for analysis before the material is released for use or formulation. Also, quality control data is obtained for all formulated material prior to its release to the clinical investigators.

It should be pointed out that the Chemotherapy Program deals with a great diversity of chemical compounds, many of which are entirely new with no methods of assay available. Very few compounds are assayed on the routine basis; thus, in many cases, methods of assay must be developed. These assay methods are made available and serve as an invaluable tool to the investigator using the particular compound. The program on analytical studies of anticancer compounds became involved in working out analytical methods for many of the compounds in biological media such as blood and urine during this report period.

In addition to the identification and analysis of compounds under study, the analytical unit is deeply concerned with those physical and chemical characteristics of the compounds which must be considered in the administration, formulation, and storage of the drug. When the unit has sufficient data to properly describe the compound, it assembles this on a Chemical Information Sheet for distribution to investigators. The solubility, stability, pKa, and methods of analysis for the drug in body fluids are of particular interest to investigators.

Among the techniques that are most commonly used in the drug analysis work are: spectroscopic determinations (ultraviolet, visible, infrared, and nuclear magnetic resonance), chromatographic determinations (paper, thin layer, and gas-liquid), and titrimetric determinations (aqueous, non-aqueous, and potentiometric). In addition to the above, elemental micro-analysis is carried out. Where applicable, optical rotation, optical rotatory dispersions, refractive index, functional group assay, and the degree of hydration or solvolysis determinations are performed. For relatively simple, stable, well-characterized compounds, a few physical properties such as melting point, infrared and ultraviolet spectra, and paper chromatography rapidly give an answer. For others, the more complex compounds, the determinations of purity and stability constitute an analytical research problem for each such compound.

Of increasing importance to the program is stability and solubility data, which serves as a guide for drug formulation, storage, and shipping. The micronization of insoluble materials to ultra-small particle size is being used as a means to the solubilizing of these compounds.

The need for the evaluation of the "shelf-life" of formulated drugs is an increasing problem. In order to establish the effective life of a drug, the continuing assay of these materials is required and is performed on a regular basis.

Another function of the quality control group is the analytical evaluation and/or certification of materials purchased on the open market. Inquiries received from scientific investigators and participating clinicians as to drug quality are also handled.

In addition, the necessary analytical data for the Food and Drug Administration are collected and assembled. Files are maintained on the drugs in clinical trial in compliance with the Food and Drug Administration regulations covering "Investigational New Drug Applications."

The Unit collects and files such data as:

1. The detailed method of preparation;
2. The suppliers analytical data sheet; and
3. The independent analytical evaluation of each lot of chemical or drug as evaluated by the Cancer Chemotherapy National Service Center-directed quality control contract.

The assembled data is of use also in writing purchase specifications for additional quantities of the compound. In general, attempts are made to keep specifications brief by listing those requirements which are judged to best identify and assay the compounds.

During the calendar year 1967, 84 compounds, representing 102 different lots of bulk chemicals and 71 lots of clinical formulations, have been evaluated. Each of these evaluations is treated individually, and the extent of analysis for each sample is a matter that is jointly decided by the Drug Development Branch and the contractors. The analytical data occasionally indicates unacceptable materials resulting in their being returned to the manufacturer for reprocessing.

In addition, several special studies were performed. Assistance was also extended to the formulation facilities with their analytical and formulation problems concerning compounds of interest to the Chemotherapy Program.

The analytical investigations are conducted under contract with Stanford Research Institute. The technical abilities available at Stanford Research Institute are of the highest calibre. Many problems are resolved through the application of instrument studies. Other problems may be approached by more sophisticated techniques. The elemental analyses are obtained from Micro-Analysis, Inc., under a purchase order arrangement.

In June, 1967, a contract was initiated with the University of Pittsburgh (Contract No. PH43-67-1186) for the determination of the structure via x-ray crystallographic techniques. The project is limited to the study of materials whose structures are otherwise difficult to establish and which are of significant interest to the program. Thus, elucidation of new structures will permit comparisons with known active agents, allow establishment of structural criteria for antitumor activity, suggest unique synthetic departures in design of analogs, and provide the basis for including selectivity in assays in drug quality control.

The contract has been valuable in substantiating, through direct comparison of single crystal x-ray data, the identity of rubidomycin hydrochloride (NSC-83142) with daunomycin hydrochloride (NSC-82151).

Work is also progressing satisfactorily on the structural study of the transformation product of imidazole-4-carboxamide, 5-[3,3-bis(2-chloroethyl)-1-triazeno]- (NSC-82196), a compound presently undergoing clinical evaluation. Knowledge of the structure of this transformation product would be helpful in the analysis and use of the agent.

CHEMICAL AND DRUG RECORDS SECTION

DRUG DEVELOPMENT BRANCH

Scope

The Chemical and Drug Records Section is charged with the responsibility of providing a continuous flow of compounds for the primary screen; of deriving systematic names for all compounds entering the program, and of insuring the use of proper nomenclature in all publications emanating from the Chemotherapy Program; of maintaining adequate storage and inventory of bulk chemicals, and of coordinating distribution of materials shipped to contract screeners and to other interested investigators. These functions of the Section are of vital importance to the entire cancer chemotherapy program.

Staff

The Section is staffed with six professional chemists and one biologist (M.S. and B.S. or equivalent) of which four are involved primarily with accessioning and nomenclature, and two are principally concerned with the procurement of new synthetic chemicals and drugs for the primary screen. In addition to their specific duties, these staff members also serve as project officers on various contracts that are maintained by the Drug Development Branch.

Acquisition of Compounds for Primary Screening

The majority of compounds received by the Service Center are voluntary contributions from commercial sources, universities and other non-profit organizations (including research institutes), government agencies, and interested individuals.

Compounds are submitted by university professors who desire to uncover chemotherapeutic agents for cancer, and by National Institutes of Health grantees who make use of our service for testing possible antitumor active compounds.

The success of the voluntary support of the Cancer Chemotherapy National Service Center is dependent upon the wide acquaintance of and the continual efforts towards good public relations on the part of the Drug Development Branch staff members. Liaison must be maintained between the Drug Development Branch and any potential supplier in order to insure the interest and continuing cooperation of the contributors. These relationships are maintained by correspondence and by personal contact.

It is often found that university professors have accumulated large supplies of research samples from past research programs. When the existence of these collections are discovered, contact is made and arrangements to procure these materials are made. The Chemistry Departments of several universities were visited in the past year. As indicated in the following table, the number of compounds obtained from these visits represents a large in-put of new synthetic materials into the program.

COMPOUNDS OBTAINED FROM COLLECTION TRIPS

Number of universities visited	27
Total number of compounds acquired	1536
Total number of new compounds	1176

In addition, one collection trip to a pharmaceutical house resulted in 740 samples collected, of which 518 were new to the program. The total number of samples obtained from collection trips is 2276 for which 1694 were assigned new accession numbers. Roughly 30% of the compounds collected in this manner were duplicates. For the calendar year of 1967, 7413 compounds were accessioned; of which 875 compounds were prepared under contract, 237 compounds (3.3%) were purchased, 2773 compounds (37.6%) were voluntary submissions from industrial sources, and 3528 compounds (47.3%) were from non-industrial sources.

It has been estimated that about four million different organic chemical compounds have been published in the literature, including 21,300 different ring structures. Of this vast reservoir of compounds, the cancer chemotherapy program has received about 130,000 compounds (3.3%) including representatives of 1361 different ring structures (6.4%). Active compounds are included in 247 of the ring systems.

Chemical Accession and Nomenclature

The Chemical Accession and Nomenclature Unit is responsible for the accessioning of all compounds entering the Cancer Chemotherapy National Service Center program and the assignment of correct nomenclature based on Chemical Abstracts and/or the International Union of Pure and Applied Chemistry systems of naming. The Unit functions as a service unit to all areas of the chemotherapy program where questions of nomenclature are involved.

Over 11,200 synthetic compounds have been processed during the calendar year through this unit, of which approximately 30% were duplicates. The duplicate samples have been used as supplementary material for any additional evaluation required by the program.

At the request of the Publication Section, Program Analysis Branch, the Unit reviewed the nomenclature of compounds in 25 articles approved for publication in Cancer Chemotherapy Reports. In addition, the nomenclature of compounds entering the data processing system and published in the supplement to Cancer Research was also reviewed.

The Unit is responsible for maintaining the confidential status of compounds received from industrial sources. The recognition by these organizations that the Branch maintains constant surveillance over commercial discreet materials has afforded not only a good industrial relationship but has made available many commercial samples not otherwise accessible.

The Unit often requests the release of compounds from confidential status. Declassification is achieved only when the industrial supplier agrees in writing to declassify his compound.

Chemical and Drug Distribution

The Chemical and Drug Records Section is also responsible for the receiving, storage, and distribution of chemicals for screening. The Section is charged with the custody of bulk chemicals and drugs that enter the program. Detailed inventory records, such as--source, date received, amount, lot numbers of samples, initiator of all shipments, and destination of drugs--are recorded on a daily basis.

The Section directs a service contract (PH43-65-587) with Microbiological Associates, Inc., which handles the receiving, weighing, record-keeping, distribution, and storage of compounds. Over 100,000 different materials are held in storage. These consist largely of chemicals and, to a lesser extent, plant products, antibiotics, and steroids.

During 1967, approximately 2100 packages were shipped by the contractor to various investigators throughout the world. Approximately 7000 new compounds and 7000 refills were weighed and packaged for shipment. On the average, the unused portion of 1200 compounds per month have been returned by screening contractors. The compounds, for which testing has been completed, are weighed and logged into the inventory.

CLINICAL DRUG DISTRIBUTION SECTION

DRUG DEVELOPMENT BRANCH

Scope

The Clinical Drug Distribution Section is responsible for the pharmaceutical activities of the Cancer Chemotherapy Program. The primary objective is to provide the clinical program with formulated drugs of uniform quality. By means of contract facilities and commercial sources, chemotherapeutic materials are prepared in proper pharmaceutical dosage form to meet rigid specifications in accordance with the criteria established by the Drug Development Branch and the Food and Drug Administration requirements relating to investigational compounds.

The responsibilities of the Section can be categorized as follows:

1. Development of new dosage forms for clinical programs;

2. Procurement of clinical dosage forms;

3. Quality control of all clinical dosage forms; and

4. Storage and distribution of clinical dosage forms.

Staff

The present staffing of this Section consists of three Pharmacists, two shipping and receiving clerks, and one clerical secretary. One Pharmacist is assigned to the Pharmaceutical Development Service, Pharmacy Department, Clinical Center, NIH.

Development of New Dosage Forms for Clinical Programs

Following the selection of a material as a candidate for clinical trial, preclinical toxicological studies are carried out to determine maximum tolerated dose levels in several species. Simultaneously with these studies, consideration is given to the development of a dosage formulation by the Section. Occasionally, when the available chemical data indicate limiting solubility and stability properties in the most common physiologically accepted vehicles, pharmaceutical development studies are requested prior to the initiation of the toxicological studies.

The first consideration of any development project consists of a review of structural, physical, and chemical data to aid in circumventing possible formulation problems. Then, a review of the preclinical *in vivo* activity data is made to determine the active route of administration and the anticipated dosage level. This information is usually sufficient to proceed with development studies leading to a suitable clinical dosage form.

This Section accumulates the chemical, physical and animal data available and prepares a "development project" for assignment to one of the pharmaceutical laboratories under contract with CCNSC. In preparing the "development project," the Project Officer frequently advises his contractor of the best probable experimental approach to the project.

Drugs considered for parenteral formulation are subjected to solubility studies in a selected group of physiologically accepted solvents. The drug solutions are then studied for the effects of pH, temperature, light, and atmospheric oxygen. Additional compatibility testing is performed with buffers, preservatives and vial closures. Drugs considered for oral use are studied for compression properties, compatibility with binders, photo-degradation and temperature effects.

Several experimental formulations are prepared and subjected to a minimum of six weeks of stability studies under accelerated conditions by which an Arrhenius plot can provide theoretical shelf-life stability data. Analytical methodology is developed and checked for effectiveness by intentional degradation of the drug with subsequent identification of the degradation products where possible. Dr. Peter Lim of Stanford Research Institute has assisted with the analytical development of several compounds.

At this point, toxicological results, maximum tolerated dose levels, and effective drug routes are reported to the Clinical Drug Distribution Section. These data and the accumulated pharmaceutical development data are submitted to members of the Dose Formulation Committee as a basis for recommending a dosage form for Phase I clinical trials.

The dosage form recommended by the Dose Formulation Committee is submitted for in vivo activity studies, comparing the antitumor activity of the original bulk drug with that of the new clinical formulation. The final dosage form is also sent to an independent laboratory for chemical and/or biological analysis. The manufacturing and control data are then accumulated and organized for review by the Quality Control Committee for Chemicals and Drugs.

This Section compiles the necessary manufacturing and control data on new dosage forms for Investigational New Drug Applications. This information, after review by the Quality Control Committee for Chemicals and Drugs, is made available to the appropriate reviewing group for filing with the Food and Drug Administration. The Clinical Drug Distribution Section assisted in the submission of 8 Investigational New Drug Applications and 37 amendments to existing IND exemptions.

Procurement of Clinical Dosage Forms

Most of the dosage forms used in the clinical program were formulated under contract. These contractors are capable of providing pharmaceutical pilot plant operations for development and production of parenteral and/or oral products of suitable quality for clinical investigation. Production assignments are initiated after a dosage form has been recommended by the

Dose Formulation Committee for initial Phase I clinical trial. In addition to the original formulation of new compounds, production assignments are initiated for new dosage forms of previously formulated compounds and for the maintenance of stock supplies of "on-going" clinical protocols. Table VII illustrates the new compounds formulated and the new dosage forms produced during 1967.

TABLE VII

NEW CLINICAL DOSAGE FORMS PRODUCED DURING CALENDAR YEAR 1967

NSC NO.	DRUG	DOSAGE FORM
740	Methotrexate	0.8 mg/tablet
749	8-Azaguanine	250 mg/vial
10023	Prednisone	100 mg/tablet
12198 E	Dromostanolone propionate	50 mg/ml/vial
17256 E	Pregn-4-ene-3,11,20-trione, 6α-methyl-	200 mg/tablet
26198 E	Dromostanolone	50 mg/tablet
26271	Cyclophosphamide	30 mg/tablet
29630	Dichloromethotrexate	250 mg/vial
36091 E	6α-Fluorotestosterone propionate	50 mg/ml (vial)
38297	5-Bromodeoxyuridine	0.5 gm/vial
45388	Imidazole-4(or 5)-carboxamide, 5(or 4)-(3,3-dimethyl-1-triazeno)-	100 mg/capsules 200 mg/capsules 100 mg/vial
50908 E	Etiocholanolone	10 mg/ml (vial)
51095	6-Trimethylammoniumpurine chloride	0.5 gm/vial
56410	Porfiromycin	15 mg/vial
63878	Cytosine arabinoside hydrochloride	100 mg/vial 500 mg/vial
65346	Sangivamycin	8.0 mg/vial

NSC NO.	DRUG	DOSAGE FORM
68626	1-acetyl-2-picolinoylhydrazine	250 mg/tablet
69948	19-Nortestosterone, 7α-methyl-, acetate	16.5 mg/ml (vial) 50 mg/ml (vial)
77385	Sodium fluoride	50 mg/tablet
81408	Testosterone, 4,5α-dihydro-2α-methyl-, tetrahydropyran-2-yl ether	100 mg/capsule
82196	Imidazole-4(or 5)-carboxamide, 5(or 4)-[3,3-bis(2-chloroethyl)-1-triazeno]-	30 mg/capsule
85998	Streptozotocin	100 mg/vial
88536 E	17β,17α-Dimethyltestosterone	50 mg/tablet
93236	16-Oxaestra-1,3,5(10)-trien-17-3-hydroxy-	100 mg/tablet
109229	L-Asparaginase	2000 I.U./vial

The Clinical Drug Distribution Section monitored three contracts for development and production of clinical dosage forms (see Table VIII).

TABLE VIII

DRUG FORMULATION CONTRACTORS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Charles Pfizer and Co., Inc.	Carleton	PH43-67-687
Burrough Bros. Pharmaceuticals, Inc.	Schlingman	PH43-65-2
Ben Venue Laboratories, Inc.	Wickes	PH43-66-909

An operation for a limited number of specialized items and pharmaceutical services is maintained at the Pharmaceutical Development Service of the Pharmacy Department, Clinical Center, NIH. The Pharmacy is provided with a full-time Senior Assistant Pharmacist to support this work. This operation has proved most beneficial in emergency situations where experience and close staff supervision were necessary. The Pharmaceutical Development Service was assigned 17 formulation projects and has delivered 5960 vials and 3000 tablets. All projects assigned required preliminary research and development, which resulted in resolution of difficult formulation problems at substantial economical savings to the program.

Pharmaceutical manufacturers supplying compounds for eventual clinical trial are encouraged to develop their own dosage forms. Many of these compounds are supplied under "prep lab" contracts. The specifications for formulation and production are provided to the contractor by this Section. Such an agreement has proved economical, expedient, and extremely beneficial. The following companies have participated in these efforts.

CONTRACTOR	CONTRACT NO.
Bristol Laboratories	PH43-64-1159
The Upjohn Company	PH43-62-168
The Upjohn Company	PH43-64-508
E. R. Squibb and Sons	PH43-65-1056

An overall production achievement of 14,592 vials and 20,000 capsules was realized by utilizing the above facilities.

The total production achieved from formulation contracts, Pharmaceutical Services, and other contract means, accounted for the following:

Vials	203,705
Capsules	220,360
Tablets	1,845,580

The Section also procures commercially available dosage forms for clinical distribution. These drugs are purchased either through the Veterans Administration Supply Catalogs, or by special purchase through negotiated contract mechanisms. A total of 126,486 vials and 10,650 bottles of tablets were purchased during calendar year 1967.

Quality Control of All Clinical Dosage Forms

The quality control program is designed to insure that each batch of formulated material meets the criteria for strength, quality, and purity. Parenteral products are tested for sterility, safety, pyrogenicity, etc.; oral products are tested for weight variation, hardness, disintegration, etc. All products are subjected to a minimum of 5 individual assays for potency depending on the dosage form and the size of the production batch. All new dosage forms are submitted to an outside analytical contractor for a confirmatory analysis. This procedure is followed to verify the analytical methodology and accuracy of the formulator. Most of the confirmation analyses are performed by the Stanford Research Institute.

This Section also maintains a shelf-life surveillance program on all lots of non-commercial formulated dosage forms. Representative samples of each formulated lot are stored under specified controlled conditions and assayed for chemical and/or physical change. Two assay schedules are used for shelf-life studies: Schedule A, designed to provide stability data under accelerated conditions; and Schedule B, designed for controlled studies of repeat formulations where adequate stability data is available. Both schedules provide a two-year surveillance record.

STORAGE AND DISTRIBUTION OF CLINICAL DOSAGE FORMS

In accordance with the provisions of Section 505(i) of the Federal Food, Drug and Cosmetic Act and S130.3, Title 21, of the Code of Federal Regulations, a perpetual inventory control system is required showing the investigator to whom shipped, date, quantity, and batch or code mark of each such shipment and delivery. This record is to be retained for "two (2) years after shipment and delivery of the drug is discontinued and the Food and Drug Administration has been so notified."

During 1967, the Section has maintained an average inventory of 270 dosage forms by lot number, involving 166 different formulations of 91 different compounds. During 1967, a total of 320,815 drug units (vials or bottles) were received and inventoried for subsequent world-wide distribution. During that same period 281,655 drug units were distributed averaging 22 individual shipments daily.

This Section is also responsible for coding of drugs for double-blind studies. These studies may involve two or more drugs; the same drug in different dosage strengths, or a combination thereof. In all instances, these dosage forms are indistinguishable in appearance for double-blind purposes. The codes are placed on each bottle or vial by the supply clerk and checked by the Pharmacist prior to shipment. There are presently 23 such coded studies on inventory.

Another phase of the Clinical Drug Distribution program involves the management of returned drugs from clinical investigators. The returned drug information is recorded on the original inventory Kardex file including the name of the investigator, the amount of drug returned and the date received. This information completes the distribution cycle of the drug since the original shipping data is also on the same card by investigator's name, quantity shipped, etc. The disposition of the returned drug is dependent upon the condition of the drug container, the age and known stability of the dosage form, the amount of drug in question and other factors. During 1967, 23,913 returned drug units were received and recorded.

NATURAL PRODUCTS SECTION

DRUG DEVELOPMENT BRANCH

Scope

This Section has as its primary objective the development of novel cancer chemotherapeutic agents from microbial sources, higher plants, and animal products. These areas will be discussed under the headings Fermentation Products Program and Plant and Animal Products Program.

Staff

This Section operates with a staff of four persons, consisting of two professional (Ph.D. level) and two clerical staff.

Fermentation Products Program

Fermentation products represent an important part of the search for and development of new chemotherapeutic agents. Research has shown two groups of organisms, the actinomycetes and the fungi, to have remarkable biosynthetic capabilities. Compounds isolated from these microorganisms have demonstrated a wide range of biological activities and chemical structures. Such novel structures may also provide the opportunity for further chemical alterations.

The Fermentation Products Program consists of two distinct but related parts. They are: (1) the search for antitumor agents and (2) the search for antimicrobial agents for supportive therapy.

(1) Antitumor Fermentation Products

This program has been carried out through contracts at a number of different biological laboratories, each of which uses its own approach to soil collections, culture isolation, selection of culture types, fermentation development, and chemical isolation. Some of the contracts in this area differ from the synthetic and plant products contracts in that a sizable amount of the effort is devoted to in-house screening and other assay services. The program supports five contracts in this area.

TABLE IX

CONTRACT LABORATORIES FOR FERMENTATION PRODUCTS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Bristol Laboratories	Bradner	PH43-64-1159
Michigan Department of Health	Olson	PH43-65-7
Charles Pfizer and Co.	Oleson	PH43-68-45
E. R. Squibb and Sons	Berk	PH43-65-1056
Upjohn Co.	Fonken	PH43-62-168

Abbott Laboratories, Brooklyn Botanical Garden, Parke, Davis and Co., Schering Corporation, U. S. Department of Agriculture (NURDD), and Wyeth Laboratories have also participated in this program in the past.

Bristol, Charles Pfizer, and Squibb have selected primarily the actinomycetes for development and to a lesser extent studied other types of microorganisms. Each company has selected colonies according to its own techniques and criteri thus resulting in a diversity in the type of chemical products produced.

All cultures formerly confirmed against the S-180, Ca-755, C-1498, LL, and Duo I tumor systems in vivo and against the KB, ILB, and Rouse Sarcoma virus cell systems in vitro have now been retested for activity against the LE and WM in vivo systems. Only those showing activity in one of these systems are being retained for further study. Old and new cultures active against the ILB or the special biochemical in vitro systems are concentrated approximately ten-fold and retested for LE and WM activity before being dropped from further study.

A critical review of the types of organism studied for antitumor antibiotic production over the past eight years shows that the fungi have not received extensive study while the actinomycetes have received the greater emphasis. Contractors are being urged to include more fungi in their new culture selections and to secure more genera and species not previously studied for biological activity.

The statistical summary of the Fermentation Products Program for 1966 and 1967 is shown in Table I.

Pure Streptozotocin (NSC-85998) and L-Asparaginase (NSC-109229) were placed in clinical trial during this contract year but the results and conclusions on these materials are not yet available. The toxic dose in man does not appear to correlate with the toxic dose in animals. This questionable relationship will receive further study in both species.

Neocarzinostatin (NSC-69856) has been formulated for clinical study. Toxicological studies are not yet completed but it is planned to place this material in clinical trial in the near future. This antibiotic is active in L-1210 and has a broad therapeutic range of activity.

Another antibiotic, Nogalamycin (NSC-70845), was being groomed for clinical trial but has been dropped due to toxicological problems.

5-Azacytidine (NSC-102816) possesses high L-1210 activity and is of considerable interest. Procurement has been difficult due to the high cost, but improvements in synthesis have markedly lowered the cost. The Upjohn Company continues to study production of this compound by fermentation and is searching for other economic sources.

Work is continuing to secure additional representatives of the "anthracycline" group of compounds related to Daunomycin. Rubomycin, obtained from Russia, has been received and chemical studies indicate it is essentially identical to Daunomycin. Samples are, however, less pure than Daunomycin. *In vivo* testing of Rubomycin is continuing. Samples of Galirubine A and four different aglycones were received from Germany but quantities were not sufficient for testing. Attempts to secure the organism producing these materials, for the purpose of preparing larger quantities, has been unsuccessful.

The antibiotic literature is continuously scrutinized for new compounds which would be desirable for screening against *in vivo* tumor systems. As a result, a total of 23 new antibiotics were requested and received for study in the past year from sources outside of our contract program.

(2) Antimicrobial Agents

Since most cancer chemotherapeutic agents are either leukopenic or immune-suppressive, the prevention and/or treatment of secondary infections has become an important area in the chemotherapy of cancer. As a guide to the search for new chemotherapeutic agents, the following rating of invading organisms has been suggested and is arranged in descending order of the severity of the clinical problem:

1. *Pseudomonas*

2. *Candida*

3. *Proteus*

4. *Klebsiella* - *Aerobacter*

5. *Escherichia*

6. *Aspergillus*

7. *Streptococcus*, Type D (Enterococci)

8. *Staphylococcus*

9. *Nocardia*

A review of published data on antibiotics has indicated a number of agents which should receive study. The quantity of material available continues to present a problem. When compounds are not available and the published data is of sufficient interest, an attempt is made to secure a culture of the producing organism. This is then submitted to one of our contractors for production of a quantity of the antibiotic for thorough evaluation. Ten antibiotics, as pure materials, have been obtained from the U. S. and other countries for testing in a screen operated by the Drug Evaluation Branch. Nine of these ten have shown activity in our screen and will receive further study.

Plant and Animal Products Program

In carrying out its objectives in the plant and animal products area, the Section has developed programs, chiefly under contract, but also to a small extent with collaborating research grantees and other organizations in (1) plant (and more recently, animal) collecting, (2) preparation of extracts, (3) fractionation of active extracts and isolation and identification of the active agents, and (4) preparation of large amounts of active agents for preclinical toxicology and clinical trial. Also, the Section searches the published literature for known constituents of active plants and attempts to acquire them for testing. Those compounds already in the CCNSC program are scheduled for additional testing, if the data indicates this is desirable. The Section through written correspondence maintains contact with domestic and foreign suppliers in order to acquire plants, animals, and pure compounds of natural origin which are of special interest to the Program. In addition, this correspondence provides information relating to the use of plants and plant products in medicine (popular, native, and folklore).

Because of the increasing necessity in recent years to re-collect large amounts of natural products for fractionation, the general collection of new plants up to last year has substantially decreased. Steps taken last year to broaden and strengthen this phase of the program were successful in providing a record number of materials for the year under review.

Progress was made in the exploration of new areas of natural products, as follows:

(1) Algae. Two contracts were established last year--with IIT Research Institute and with Tyco Labs., Inc. These were concerned with the cultivation of algae in pure culture and preparation of aqueous-ethanolic extracts of the cells and of the filtrates. Extracts are screened under other CCNSC contracts. While no active materials have yet been obtained in this work, it should be noted that too few materials have yet been produced and too little testing is yet in hand for a reasonable expectation, on statistical grounds, of positive results. Only 63 species of algae have been grown, extracted, and sent in for test. One of the two contracts (Tyco) has been renewed for another year. The two years should give us sufficient opportunity to judge whether the use of algae is worth pursuing further.

(2) Marine invertebrates. Pfizer's work with the sponges has yielded many active materials but none as yet has been isolated in pure form or in sufficient quantity for evaluation as a potential drug. The coming year should see notable advances in this field. The contract established last year with the University of Miami has not been in operation long enough to yield any positive results; it has been renewed for another year. In addition, we have developed informal contracts with other individuals who have supplied extracts of a number of invertebrate species. Thus, Dr. Seabra, the University of Rio de Janeiro, has sent extracts of a number of organisms

from the waters off northern Brazil, and Dr. Weinheimer, the University of Oklahoma, has provided extracts of a number of species largely from the Bahamas. Again, numbers of extracts have been too few to date to expect positive results out of this work. In an attempt to put this area on a firmer basis statistically, contract proposals were recently solicited. These are presently under evaluation and, hopefully, the next year will see more work in this field.

(3) Insects. The contract with Butterfly Art Jewelry, Inc., mentioned in the last Annual Report did not materialize due to administrative difficulties. However, we were able to purchase over 100 species of a variety of insects from this company, and many other species from other commercial insect suppliers. Extracts were prepared by Dr. Pettit, Arizona State University, working under an NIH research grant. In addition, Mr. Jacobson, Pesticide Chemicals Research Branch, U.S.D.A., has been supplying us with several species for testing.

One of Mr. Jacobson's extracts has shown confirmed activity in one of our tumor systems and is under fractionation. Several of Dr. Pettit's extracts have shown preliminary activity and it is expected that confirmatory data will be obtained on some of them. It is interesting to note that oncolytic activity is not restricted to extracts of butterflies alone but is found also in extracts from a moth, a beetle, and a grasshopper, and there are indications that it may be found in extracts from an ant and a dragonfly. From the source of the extracts and the method of preparation, it seems unlikely that activity would be due to the presence of tannin or β-sitosterol as is the case with so many plants. It is hoped that new classes of active agents will emerge from this work.

In addition to the above, the Plant and Animal Products Program has continued to apply the techniques described in last year's Annual Report designed to eliminate unpromising plant extracts from the list of confirmed active plants and thus retain a smaller but more selective list of plants for re-collection and fractionation.

The following contractors are engaged in the Plant and Animal Products Program:

TABLE X

PLANT AND ANIMAL PRODUCTS CONTRACTORS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Arizona, Univ. of	Brewer; Cole	PH43-67-1484
Costa Rica, Univ. of	Saenz R.	PH43-64-31
Commonwealth Scientific and Industrial Research Org.	Culvenor	PH43-64-522
Hawaii, Univ. of	Sagawa	PH43-67-748
IIT Research Institute	Shefner; Gross	PH43-67-724

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Miami, Univ. of	Sigel	PH43-67-1179
Charles Pfizer and Co.	Oleson	PH43-68-45
Research Triangle Institute	Wall	SA-43-ph-4322
Tyco Laboratories, Inc.	McLeod	PH43-67-723
U.S. Department of Agriculture	Perdue	NCI-CR-64-1 (Transfer)
Wisconsin Alumni Research Foundation	Derse	PH43-67-747
Wisconsin, Univ. of	Kupchan	PH43-64-551

Besides the contractors listed above, certain major investigational groups contribute to the Plant and Animal Products Program.

1. Central Drug Research Institute, Lucknow, India, collects native Indian medicinal plants, supplies extracts therefrom, and fractionates the active extracts under a PL480 agreement, for screening by CCNSC. At present, 16 plants are under fractionation.

2. The University of Brazil, Rio de Janeiro, collects native Brazilian plants and prepares the corresponding extracts for screening by CCNSC. Recently, a few extracts from marine invertebrates have been provided.

3. Smith Kline and French Laboratories, Philadelphia, Pennsylvania, is supplying, under purchase order, extracts of plants collected from all over the world. During 1967, 1335 extracts were supplied. As the confirmed active plants are re-collected they are assigned to our collaborating chemists for fractionation.

4. Besides its contract work, the Commonwealth Scientific and Industrial Research Organization (C.S.I.R.O), Melbourne, Australia, collects native plants and sends them to CCNSC contractors for extraction and screening. At present, extracts from 104 species of plants have shown reproducible antitumor activity. Many have been re-collected in quantity, and 53 are, or have been, under frationation. C.S.I.R.O has not been able to fractionate all these plants in its own laboratories and has arranged to have some of the work done by 17 collaborators in Australian university laboratories and other government laboratories.

5. Mr. Martin Jacobson, Pesticide Chemicals Research Branch, U.S. Department of Agriculture, Beltsville, Maryland, is fractionating several active plants under the guidance provided by our bioassays. One of the insect extracts, recently provided, has proved to be active and also is under fractionation.

6. Dr. Ivan A. Wolff, Northern Utilization Research and Development Division, U.S. Department of Agriculture, Peoria, Illinois, is also fractionating several active plants under the guidance of CCNSC bioassays.

7. Several NIH research grants are devoted to the isolation of natural products from plants and to the study of their pharmacological properties. In some cases, CCNSC performs the bioassays necessary to guide the fractionation of these plants.

8. Arrangements have been made with several individuals to receive and test either plants or their extracts. These collections are usually from a special locality.

A large number of active agents were isolated from confirmed active plants. Of those that were not duplicates of compounds already accessioned by CCNSC, several were isolated in large enough amounts for evaluation. Table I summarizes the numbers of extracts and agents produced.

The four new compounds accepted for toxicological studies were ellipticine (NSC-71795), β-sitosterol carboxymethylthiosuccinate (NSC-110372), protein from Caesalpinia gilliesii (NSC-110435), and acronycine (NSC-403169).

In addition, during the year under review, and since January 1, 1968:

1. The list of known constituents of active plants (obtained from literature surveys) has been continuously updated. This list is used to determine which of these constituents have already entered the CCNSC screening program and if any new screening should be scheduled.

2. Crude extracts of plants with confirmed activity against tumor systems other than WM or LE have continued to be re-tested against these two systems and those that have not shown activity have been dropped from present consideration.

3. The two techniques developed last year for increasing the efficiency of the fractionating step, i.e., the tannin procedure for SA- and WM-actives and the "standard fractionation" for KB-actives, have continued to be utilized rather fully and have completely justified their existence by enabling a large number of active plants to be eliminated from further consideration both for re-collection and for fractionation.

4. The literature and folklore survey of anticancer plants, under preparation for about fifteen years by Dr. Hartwell, has been completed and the final typing of the large manuscript is nearly finished. The work is to be published serially in Lloydia. The first installment appeared in the December, 1967 number under the title "Plants Used Against Cancer. A Survey."

Bulk Procurement

The preparation of large amounts of Step IVA agents from natural products has presented special problems of procurement and extraction which were indicated in past Annual Reports. At the present time, bulk collections of all plants required for the preparation of Step IV compounds of plant origin are in hand and isolation is either in process or completed. These are, in summary:

PLANT	STEP IV AGENT
Camptotheca acuminata, Calif., 936 lbs.	Camptothecin (NSC-94600) Camptothecin sodium deriv. (NSC-100880)
Thalictrium dasycarpum, Wis., 3700 lbs.	Thalicarpine (NSC-68075)
Acer negundo, Utah, 11,000 lbs.	Saponin (NSC-100045)
Cyclea peltata, India, 6000 lbs.	Tetrandrine (NSC-91771)
Synthesis [1], 5 kg	Ellipticine[1] (NSC-71795)
Tabebuia sp., Dutch Guiana	Lapachol (NSC-11905)
Caesalpinia gilliessii, Ariz., 145 lbs.	Protein (NSC-110435)
Acronychia baueri, Australia	Acronycine (NSC-403169)

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[1] Originally obtained from *Ochrosia moorei* and *Excavatia coccinea* but these sources were not plentiful enough for bulk preparation.

Of these agents, lapachol is in Phase I clinical study; the sodium derivative of camptothecin and ellipticine are undergoing toxicological evaluation, and are in dosage formulation development; thalicarpine is ready for toxicology; and the others are nearing completion of their large-scale preparation.

ANNUAL REPORT

DRUG EVALUATION BRANCH

CANCER CHEMOTHERAPY NATIONAL SERVICE CENTER

CHEMOTHERAPY
NATIONAL CANCER INSTITUTE

I. Objectives. Principal objectives of the Drug Evaluation Branch (DEB) are to initiate, develop, and coordinate laboratory programs for (a) recognition and evaluation of new potential anticancer agents, (b) detailed investigation and development of agents of potential clinical interest, and (c) the recommendation of new drugs for preclinical toxicology and clinical trial. To achieve its objectives, the DEB has developed programs for (1) primary screening of large numbers of synthetic and natural products for activity against experimental tumors, (2) detailed evaluation with respect to optimal conditions of treatment (dosage, schedule, route, etc.) of materials which display "activity" in primary screens, (3) studies of drug effects on host defense mechanisms and their implications to chemotherapy, (4) studies of mechanisms of drug action and resistance, (5) the development of new and improved experimental methods of drug evaluation, (6) laboratory investigation of problems related to drug evaluation that may arise during preclinical toxicological evaluation or clinical studies, and (7) assimilation, organization, and summarization of laboratory results of pertinence for presentation to Chemotherapy Staff and clinical cooperative groups.

II. Organization. The DEB comprises the Office of the Chief and three sections; Screening, Biochemistry, and Immunotherapy and Immune Response Modifiers, and is staffed by nine senior professionals, including one Research Associate, three junior professionals, and seven statistical and clerical personnel. The principal investigators are John M. Venditti (Branch Chief), Betty J. Abbott (Head, Screening Section), Florence R. White (Head, Biochemistry Section), and John P. Glynn (Head, Immunotherapy and Immune Response Modifiers Section). Other senior investigators are David Abraham (CO), Mario C. Baratta (CO), Alexander Fefer (RA), Ruth I. Geran, and Mary M. Macdonald.

III. Implementation of Program. The DEB program is carried out largely through carefully guided contracts with universities, biomedical research centers, pharmaceutical companies, and private research organizations. The DEB staff administers directly 14 contracts funded at approximately $3,365,000 (Table 1, part I); coordinates drug evaluation activities under 6 contracts funded at approximately $5,227,000 and administered under the Office of the Scientific Director for Chemotherapy, Office of the Associate Scientific Director and Chief, CCNSC, or the Drug Development Branch, CCNSC; (Table 1, parts II - IV); and selects the natural products which are to be subjected to fractionation and isolation studies under 6 contracts funded at approximately $545,000 and administered by the Drug Development Branch, CCNSC (Table 1, part V). The DEB staff supervises the work of the Drug Evaluation

Division and Immunology Division, Microbiological Associates, Inc. under contract no. PH43-64-911. The latter projects represent a direct extension of the research program of the DEB (see this report, Serial Nos. NCI 3576, 3577, and 3578).

This report summarizes the DEB approaches toward the overall objectives of the Chemotherapy Program, NCI. Progress in attaining objectives is described more fully in (a) current organization reports of each Section of the Branch, (b) current Individual Project Reports enumerated above, (c) progress reports from contract laboratories, and (d) reports from the DEB and contract laboratories to Clinical Task Forces sponsored by the Chemotherapy Program and to the Chemotherapy Staff.

IV. Staff Functions. The DEB staff serve as Project Officers for the 20 contracts listed in Table 1, parts I and V and Assistant Project Officers for the 6 contracts listed in Table 1, parts II - IV.

Specifically, the staff (1) recommends new projects and reviews existing projects in view of the overall objectives of the Chemotherapy Program, NCI, (2) guides, instructs, orients, and reviews progress in contract laboratories, (3) recommends, designs, develops, and establishes new and improved laboratory methods for drug evaluation, (4) designs and coordinates detailed investigations of drugs of clinical interest, (5) prepares scientific reviews of accomplishments and their implications for presentation to the NCI Program and contract reviewing mechanism, and for publication, (6) organizes and analyzes experimental results, recommends new agents to the Chemotherapy Staff for clinical trial, summarizes and presents to Cooperative Clinical Groups and Task Forces information on drug efficacy and safety.

V. Accomplishments and Significance to the NCI.

A. Primary Screening. During calendar year 1967, 7,413 new synthetic agents and 11,681 natural products were subjected to the initial stages of screening. An analysis of this activity with respect to the numbers of materials screened in the various *in vivo* and *in vitro* test systems is presented in the current report of the Screening Section, DEB. The overall program for screening was based on studies of existing correlations between drug activity in experimental systems and usefulness in the clinic, and test systems utilized for the routine screen were selected following their evaluation as a "model" for predictability for clinical usefulness and feasibility for large-scale screening. Principal features of the program in 1967 were:

1. *In vivo* screening of all materials, to extent permitted by the quantity, in three phases beginning with testing as a single treatment at 3 or 4 dosage levels in mice with ascitic leukemia L1210 (LE), followed by the testing by daily treatment in the LE system, followed by testing against intramuscularly inoculated Walker 256 carcinoma in rats (WM).

2. The reservation of alternative *in vivo* systems for special purposes including (a) testing of selected related agents in systems for the possible recognition of particular biological activities which may predict

for unique clinical application and to provide information on structure-activity relationships leading to the design of more useful agents, (b) testing of structurally related materials against variant tumors developed for resistance to known active drugs in order to provide leads for combination chemotherapy and information relative to mechanisms of drug action and resistance to therapy, (c) bioassay testing associated with the isolation of the active material from crude natural products that had been previously uncovered in alternative screening systems, and (d) studies related to the evaluation of alternative systems for feasibility as future primary or secondary screens.

3. The screening of synthetic agents *in vitro* only if the supply was insufficient for the conduct of the first phase of the *in vivo* LE screen and the screening of crude natural products both *in vitro* and *in vivo* to permit the use of the less expensive *in vitro* system for bioassay of fractionation products in the isolation of drugs from the active crude products. Contract laboratories engaged predominantly in primary screening *in vivo* and *in vitro* are noted in the current report of the Screening Section, DEB.

4. During late 1967, the test systems used for primary screening were reviewed after approximately two years of experience. It was decided that the WM system would be deemphasized pending the clinical evaluation of agents which were active in this system and were inactive in LE. Such agents include Dibromomannitol (NSC-94100), Lapachol (NSC-11905), a protein from *Caesalpinia gilliesi* (NSC-110435), DL-Tetrandrine (NSC-91771), *Acer negundo* saponin (NSC-100045), Thalicarpine (NSC-68075), and Antibiotic B35251 (NSC-77471). Older agents which were active in WM and inactive in LE include Streptonigrin (NSC-45383), Demecolcine (NSC-3096), Nitromin (NSC-10107), Myleran (NSC-750), Oxylone (NSC-33001), Hexamethylmelamine (NSC-13875), Dihydroxymyleran (NSC-39069), Prednisone (NSC-10023), and Chlorambucil (NSC-3088). It was further decided that the LE system would constitute the principal tumor-host system of the screen and that an additional system should be selected on the basis of its potential for uncovering drugs which are different from those selected by LE. Such a system would be employed in primary screening until a reasonable number of materials predicted by it could be evaluated in the clinic. In the selection of a tumor system to be used in conjunction with the LE system, consideration was given to the results from research projects within this program indicating that L1210 cells exhibited a relatively short doubling time (T_d) of about one half day. Associated studies suggested that a relatively high percentage of L1210 were actively engaged in DNA synthesis (S-phase) at any point in time and that a high percentage of drugs showing activity against L1210 were cell cycle stage specific in that they inhibited biochemical pathways leading to nucleic acid biosynthesis. Thus, it was considered desirable that a tumor designed to complement L1210 in the screen be one with a substantially longer doubling time. Hopefully, such a tumor would uncover new agents which may not be active against L1210 but might also serve to determine the extent of cell cycle stage specificity for materials that are active against L1210. Four transplantable mouse tumors are under study because they exhibit a relatively long apparent Td; hepatoma 129, Harding-Passey melanoma, Lewis lung carcinoma, and the B16

melanoma. Current studies of these tumors are designed to provide information on the actual generation time (T_g) of the tumor cell, the time in S-phase and their feasibility for screening (see below, "New Methods of Drug Evaluation"). Laboratories assigned to study these tumors are listed in the current Annual Report of the Screening Section, DEB.

B. Selection of Materials as Potential Candidates for Clinical Trial. Selection of new agents from the initial stages of primary screening is based on a reproducible response which is minimal but significant at a high probability level. Such "confirmed active" synthetic agents are tested further in the sensitive system modified with respect to experimental protocol and criteria of response in order to determine whether the degree of anti-tumor specificity warrants consideration as a potential clinical candidate. "Confirmed active" extracts of natural products are entered into the CCNSC program for fractionation and isolation of the active moiety which is then subjected to the later stages of screening. Upon completion of the routine stages of screening, "confirmed active" materials are reviewed by the Drug Evaluation Committee, chaired by the Chief, DEB and composed of the professional staff of the Office of the Chief and Screening Section, DEB with representation from the Drug Development Branch, CCNSC, and Program Analysis Branch, Chemotherapy. During calendar year 1967, the Drug Evaluation Committee held 25 regular meetings in addition to one special meeting with contract personnel in conjunction with the 58th annual meeting of the American Association for Cancer Research. In carrying out its primary function of reviewing screening results and presenting potential clinical candidates to the Chemotherapy Staff, the Drug Evaluation Committee, as a consequence of its membership, functions to relate overall Chemotherapy Program objectives to DEB activities and to monitor contract projects for conformance. During the last calendar year, 399 compounds were reviewed by the Drug Evaluation Committee. Fourteen compounds were recommended for preclinical toxicology and development toward clinical trial. The latter are listed in the current report of the Screening Section, DEB.

C. Detailed Evaluation of New and Old Drugs. New agents which emerge as active from primary screens and older drugs of clinical interest are subjected to detailed studies in which stress is placed on the influence of factors such as the treatment schedule and route which are known to influence drug efficacy. Last year, 70 compounds were scheduled for such studies. The compounds studied and the experimental designs for such studies are included in the current report of the Screening Section, DEB. Schedule dependency studies are designed to provide information on the influence of the number of treatments, the interval between treatments, and the duration of treatment. Parallel groups of tumor-bearing and normal animals are included in order to provide additional information on (a) the optimal schedule for maximum tumor cell destruction, (b) the optimal schedule for host recovery, and (c) the relationship between (a) and (b) as an indication of the schedule providing maximum selective toxicity against the tumor cells. In schedule dependency studies with older drugs, emphasis has been placed on those which were "active" in the LE system but have not demonstrated clinical usefulness as well as on those which have been clinically active. As a result of these studies it has been observed that Azaserine (NSC-742), a clinically inactive drug, and Methylglyoxalbisguanylhydrazone (NSC-32946) were more effective in the LE system when administered every 3 hours per 24 hours every fourth day than when given as a single daily treatment or a single treatment every fourth day. When such examples of previously unrecognized schedule dependency are noted for older drugs, toxicological data in larger animals and clinical protocols are reviewed to determine the possibility that the drug may not have been evaluated under optimal conditions for maximum therapeutic effect. In the Chemotherapy Program, regular consultations among the staffs of CCNSC, the Experimental Therapeutics Area, and the Clinical Trials Area, provide a mechanism for the integration of such information into the planning of toxicological, pharmacological, and clinical studies.

With emergence of active materials from the WM screen, an intensive effort was placed on the development of a suitable experimental protocol for the conduct of schedule dependency studies with this tumor. Such studies are limited by the short duration of treatment in the protocol for primary screening in which mice are sacrificed for tumor weight determinations four days after the first daily treatment (one day after the final treatment). The successful development of a valid assay of the effect of drugs on the lifespan of rats with W256 has permitted the conduct of more comprehensive schedule dependency studies utilizing this tumor. It has been observed that there is no necessary correlation between a drug's ability to inhibit the local tumor in the primary WM screen and its capacity for increasing the survival time of the rats. Thus, among drugs which emerged as active from the primary WM screen, some have produced extensive increases in the survival time of rats with W256 (e.g., Dibromomannitol, NSC-94100) whereas some have elicited minimal or moderate increases in lifetime (e.g., Lapachol, NSC-11905). Studies on a limited number of materials indicated that drugs which are most effective in increasing the survival time of rats with W256 are generally regarded as alkylating agents.

Compounds of clinical interest are studied with respect to mechanisms of action, mechanisms of resistance to therapy, and means of overcoming resistance to therapy. Emphasis is placed on investigations of effectiveness against drug-resistant tumor variants and biochemical sites of drug action. Studies of the effectiveness of concomitant or sequential combination chemotherapy provide information relative to mechanisms of action and resistance but are conducted principally to uncover more effective therapies. Drug combinations which have shown potentiative or synergistic effects against L1210 include: Cytoxan (NSC-26271) plus 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU, NSC-409962), Cytosine arabinoside (NSC-63878) plus NSC-409962; NSC-63878 plus 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU, NSC-79037); NSC-63878 plus NSC-26271; 6-Mercaptopurine (NSC-755) plus Daunomycin (NSC-82151); 6-Methylmercaptopurine ribonucleoside (NSC-40774) plus NSC-755; NSC-40774 plus 6-thioguanine (NSC-752); NSC-63878 plus Porfiromycin (NSC-56410); NSC-63878 plus Actinobolin (NSC-31083); and Hydroxyurea (NSC-32065) plus NSC-409962. In addition, NSC-26271 plus NSC-409962 were found synergistic against mouse leukemia P1534.

D. New Drugs in Development Toward Clinical Trial in the Chemotherapy Program, NCI. The FY 1967 Report of the Office of the Chief, DEB presented a cumulative list of 48 new and older drugs which were in development toward clinical trial. Thirteen agents currently undergoing preclinical development in the Chemotherapy Program and which did not appear on that list are shown in Table 2. Of these, all but two (NSC-109229 and NSC-403169) were initially uncovered in this program. Three were active against both L1210 and W256. Five were active against L1210. Three were active against W256. NSC-109229 was selected on the basis of activity against the radiation-induced transplantable tumor, EARAD-1 and the Gardner lymphosarcoma, 6C3HED. NSC-403169 was selected on the basis of activity against mast and plasma cell tumors such as LPC-1. Selection of NSC-71261 and 71851 was based on activity against L1210 and W256 as well as on biochemical studies conducted at Stanford Research Institute (contract 65-575) indicating that these materials were approximately twice as effective as substrates for the tumor tissue (L1210 or W256) kinases catalyzing ribotide formation than for normal host kinases.

E. Programs Devoted to Uncovering Other Biological Activities.

1. Evaluation of Antiviral and Antimicrobial Activity. A limited viral chemotherapy program under the auspices of the DEB was conducted under contract at the Southern Research Institute (PH43-63-68). During the last year, 307 materials were screened against the Friend leukemia virus, Rous sarcoma virus, Vaccinia, or mouse salivary gland virus (*in vivo*); or human cytomegalovirus, CMV (*in vitro*). Significant accomplishments on this project are summarized in the Annual Progress Report, contract PH43-63-68. This project is scheduled to be terminated during the coming year. Continuance of screening against CMV under contract PH43-65-594 at the Southern Research Institute is anticipated.

In a project conducted at Southern Research Institute under contract PH43-65-594 to investigate activity against microorganisms isolated from cancer patients, approximately 850 materials were tested *in vitro* against one or more strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas*, *Klebsiella*, *Proteus*, or *Candida albicans*. Approximately 150 materials showed initial *in vitro* activity and 33 materials were active in secondary *in vitro* screening. Experimentally-induced infections in mice were established for *Proteus*, *Pseudomonas*, *E. coli*, *Klebsiella*, and *Staph. aureus*. Nine materials have been evaluated *in vivo*. Of these, four (NSC-9369, 19893, 23519, and 87982) were active against one or more of the organisms.

F. Research Related to New Methods Development. Methodology studies in this program are concentrated at three laboratories; Microbiological Associates, Inc. (PH43-64-911), Southern Research Institute (PH43-65-594), and Arthur D. Little, Inc. (PH43-65-61).

The Drug Evaluation Division and Immunology Division at Microbiological Associates, Inc. are collaborative laboratory resources for the DEB research staff. The Drug Evaluation Division has emphasized the development and use of new and improved methods for determining the optimal conditions for drug usage with respect to treatment schedule and route, for studies of drug resistance and the effects of combination therapy, and for the investigation of new systems for primary or secondary screening. The group, in collaboration with the Chemotherapy Research Division under the same contract, has been engaged in comprehensive studies of the operating characteristics of the Lewis lung carcinoma in mice, a tumor with relatively long T_d under consideration as ancillary to L1210 in the primary screen. The DEB program, for testing against the mouse plasma cell tumor, LPC-1, is conducted under this contract. (See current report of the Screening Section, DEB; Individual Project Reports, Serial No. NCI-3576 and NCI-3578; and Annual Report, contract PH43-64-911). The primary objective of the Immunology Division is to obtain basic information related to the immunology of viral oncogenesis. Major emphasis is placed on studies of tumor-associated antigens and host reactivity to such antigens leading to the development of immunotherapeutic models for the treatment of experimental tumors (see current report of the Immunotherapy and Immune Response Modifiers Section, DEB and Individual Project Report, Serial No. NCI-3577).

The group at the Southern Research Institute (contract PH43-65-594), in addition to carrying out studies of schedule dependency, combination chemotherapy, and drug resistance, have extended to studies of the fundamental question of the kinetics associated with tumor cell proliferation, drug effects thereon, and relationships to host recovery. Such studies, pioneered using the L1210 system, were further extended to established solid tumors including plasmacytoma in hamsters, carcinoma 755 in mice, and first generation transplants of spontaneous mammary tumors in C3H mice. Such studies have included determination of the thymidine index as a measure of the percentage of cells in S-phase during a specified period of time. Currently, tumors with apparent relatively long T_d are under investigation in this project in order to determine the relationship of T_d to T_g. An *in vitro* system was developed for estimating the cell kill potential of a drug against proliferating L1210 cells and against non-proliferating L1210 cells. Limited studies have shown that drugs which inhibit cells during the S-phase of the cell cycle destroy a higher percentage of cells in the proliferating population than in the resting population, whereas drugs which are not "cell cycle stage specific" tend to have nearly equal effects against both populations. Procedures have been established for the diagnosis of advanced spontaneous leukemia in AKR mice. These studies have suggested that leukemic (AKR) cell doubling time from diagnosis to death is probably longer than during the early course of the disease. The same phenomenon is apparent with many solid tumors. This raises the question of whether the percentage of proliferating cells decreases as the total tumor cell population increases.

The group at Arthur D. Little, Inc. (contract PH43-65-61) have continued to evaluate the antileukemic (L1210) specificity of drugs in the spleen colony assay. The assay compares the surviving fraction of leukemic colony-forming cells and normal hematopoietic colony-forming cells. The system has been used extensively to compare, for active drugs, the relative antileukemic specificity of a single treatment and closely-spaced treatments. Over 100 compounds have been evaluated. A high degree of correlation has been found between the effects of therapy using the spleen colony assay and effectiveness in increasing the lifespan of mice with L1210. Recently, the spleen colony assay has been extended to the development of systems for estimating the differential responses of normal hematopoietic and drug-resistant leukemic cells to therapy. In addition, methods are being established for studying the growth kinetics of leukemia P388 utilizing spleen or liver colony assays. The ADL group has also investigated the use of spleen weight as an indication of drug toxicity in primary screening and schedule dependency studies. In pursuit of the general problem of determining the extent to which drug toxicity "masks" antitumor activity, an "Operations Research" project was instituted under this contract in collaboration with the Program Analysis Branch. Among the objectives of the latter project are the establishment of computer processible signaling devices to indicate drug toxicity and to investigate new methods for the quantitative analysis of screening and schedule dependency studies.

VI. Proposed Course of Project. The DEB will continue to carry out its major objectives related to the selection of candidate drugs for clinical trial from the large number of materials submitted for screening in the CCNSC program and the detailed evaluation of drugs which emerge from primary screens. Increased emphasis will be placed on the development of new screening systems leading to the selection of a relatively slow growing tumor to be used together with L1210 in the primary screen in order to (a) uncover agents which are inactive against L1210, and (b) aid in estimating the extent of "cell cycle stage specificity" of active agents. The development of other ancillary systems as indicators of drug effects on the kinetics of tumor and normal cell proliferation will continue. Schedule dependency studies will continue to be directed toward providing pertinent information for the design of toxicological studies in larger animals and for clinical protocols. In addition, schedule dependency studies, combination chemotherapy studies, and recent concepts related to the kinetics of cell growth will be integrated to develop methods for measuring the capacity of therapy to eradicate the tumor cell population in experimental animals. Biochemical studies will stress drug actions at biochemical sites where inhibition is known to be associated with tumor cell destruction.

The DEB will continue to give priority to informing other areas of the Chemotherapy Program of pertinent findings. In addition to continued reporting of data and recommendations to the Associate Scientific Director and Chief, CCNSC, the Scientific Director for Chemotherapy, the Operating Committee, and Program sponsored Task Forces and Clinical Trials Groups; the DEB will emphasize the reporting of information to the Chemotherapy Staff in accordance with the Program's linear array of decision points in the progression of a new drug toward clinical trial.

Table 1

Chemotherapy Program Contract Activities Totally or Partially Within Scope of Drug Evaluation Branch Staff Functions 1/

I. Contracts Administered by DEB

A. Office of the Chief

Southern Research Institute (65-594)

B. Screening Section

Battelle Memorial Institute (62-495)
Hazleton Laboratories (63-622)
IIT Research Institute (4349)
University of Miami (64-890)
Southern Research Institute (63-68)
Wisconsin Alumni Research Foundation (66-494)
University of Wisconsin (67-1126)

C. Biochemistry Section

University of Connecticut (65-1035)
Scripps Clinic and Research Foundation (64-14)
Southern Research Institute (66-29)
Stanford Research Institute (65-575)
Yale University (67-1193)
Weizmann Institute (PL-480 funds)

II. Contracts Administered by Scientific Director for Chemotherapy

Arthur D. Little (65-61)
Microbiological Associates, Inc. (64-911)
Charles Pfizer and Company (68-45)

III. Contracts Administered by Drug Development Branch, CCNSC

Bristol Laboratories (64-1159)
Upjohn Company (62-168)

IV. Contract Administered by Associate Scientific Director and Chief, CCNS

Indian Cancer Research Centre (PL-480 funds)

Table 1 (Continued)

V. Contracts Administered by Drug Development Branch, CCNSC, for Natural Product Collection and Isolation

USDA (64-1)
University of Arizona (63-1136)
University of Miami (67-1179)
Research Triangle Institute (4322)
University of Wisconsin (64-551)
Wisconsin Alumni Research Foundation (67-747)

[1] An analysis of activities carried out under each contract is given in Table III of the current Administrative Report of the Chemotherapy Program, NCI. Activities listed in the latter table which fall within the scope of DEB staff functions are: Assay testing related to natural products development (II C), Primary *in vivo* screening (III A), *In vitro* screening (III B), Detailed evaluation of new and old drugs (III C), Tumor and cell culture banks (V D), New screening methods (VII A), Biochemical mechanisms of action and resistance (VII B). The codes in parentheses in this footnote refer to coding in Table III of the Administrative Report.

Table 2

New Agents Placed in Preclinical Development, Chemotherapy, NCI (April 1967 - March 1968)

NSC No.	Name	Selection Basis
50741	β-D-Ribofuranoside, methyl-5-(2-chloroethylamino)-5-deoxy-2, 3-O-isopropylidene-, hydrochloride	L1210
52695	Phosphorodiamidic acid, N,N-bis(2-chloroethyl)-N'-(3-hydroxypropyl)-, compd. with cyclohexylamine (1:1) (cytoxyl alcohol)	L1210
71261	9H-Purine-6-thiol, α-amino-9-(2-deoxy-β-D-ribofuranosyl)-, hydrate β-(Deoxythioguanosine, hydrate)	L1210 + W256
71851	9H-Purine-6-thiol, α-amino-9-(2-deoxy-α-D-ribofuranosyl)- (α-deoxythioguanosine)	L1210 + W256
77471	Mitochromin	W256
107392	Picolinaldehyde, 5-hydroxy-, thiosemicarbazone	L1210
107455	Wyeth's antibiotic B-192,142	W256
109229	L-asparaginase	EARAD-1, 6C3HED
110435	Protein from *Caesalpinia gilliesi*	W256
111056	Carbanilide, 4,4'-diacetyl-,bis(amidinohydrazone), dihydrochloride, trihydrate (DDUG)	L1210 + W256
113233	Mitomalcin	L1210
403169	Acronycine	LPC-1
406021	9H-Purine-6-thiol-9-β-O-arabinofuranosyl-	L1210

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Serial No. NCI-3576
1. Chemotherapy, CCNSC
2. Drug Evaluation Branch
3. Office of the Chief
4. Wiscon Bldg. - Cancer Chemotherapy Laboratories, Microbiological Associates, Inc., Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Quantitative Evaluation and Detailed Study of Drugs in the Therapy of Experimental Tumors.

Previous Serial Number: NCI-3576.

Principal Investigator: J. M. Venditti

Co-Principal Investigators: A. Goldin (NIH), I. Kline, W. Waravdekar, D. Tyrer, and G. Hoffman (Microbiological Associates, Inc., Bethesda, Maryland, under contract no. PH43-64-911, Drug Evaluation Division).

The Drug Evaluation Division, Microbiological Associates, Inc. is a collaborative laboratory resource for the research staff of the Drug Evaluation Branch, CCNSC and the Chemotherapy Program, NCI. NCI professional staff participates in an advisory capacity in day-to-day planning, execution, and evaluation of the project which represents a direct extension of the research program of the Drug Evaluation Branch, CCNSC.

Cooperating Units: Office of the Associate Scientific Director and Chief, CCNSC (S. Schepartz); Office of the Associate Chief for Laboratory Research, CCNSC (A. Goldin, W. DeWys, see this report, serial no. 3575); Chemotherapy Research Division (C. Vadlamudi); Immunology Division, and Pharmacology Division, Microbiological Associates, Inc., contract no. PH43-64-911.

Man Years: Total 2
Professional 2

Project Description:

Objectives, general:

To evaluate in detail the in vivo antitumor actions of drugs which emerge as active from CCNSC primary screens; to provide information applicable to the toxicology study and clinical use of these agents, to provide information on the optimal conditions for therapy leading to the eradication of malignant disease in experimental animals, to conduct basic studies leading to the establishment of new models for in vivo drug evaluation.

Objectives, specific:

I. To determine the influence of treatment schedule and route on therapeutic efficacy.

II. To evaluate drugs for their influence on normal and tumor cell kinetics; to rank drugs according to their relative effectiveness in eradicating tumor cells; to determine optimal conditions of dose, treatment schedule, and route for recovery of normal cells as well as maximum destruction of tumor cells.

III. To evaluate sequential and concomitant combination chemotherapy for therapeutic superiority over treatment with a single drug; to investigate the influence of treatment schedule and route on the efficacy of combination chemotherapy.

IV. Employing drug-resistant variant tumors, to evaluate new agents with respect to similarities to or differences from known drugs with respect to antitumor activity; to evaluate single drug or combination therapy as a means of overcoming resistance to chemotherapy.

V. To conduct collaborative studies on specific problems relative to drug evaluation which may be referred from other Divisions within this program or from other units within the Chemotherapy Program, NCI.

VI. To provide information, where feasible, relative to the biological quality control of new formulations intended for clinical use.

VII. To conduct basic and developmental research leading to the establishment and use of new in vivo systems for screening and drug evaluation.

Methods Employed:

Studies related to I - VI above have been conducted largely in mice with leukemia L1210. Additional studies have been conducted with the Walker 256 carcinoma in rats. Basic studies related to the development of new assays have emphasized characterization and evaluation of the Lewis lung mouse carcinoma as a potential screening tool. In general, standard laboratory procedures and new procedures developed in this program are employed in the conduct of studies.

Major Findings:

I. Influence of Treatment Schedule and Route on Effectiveness Against Mouse Leukemia L1210.

Schedule dependency studies were conducted for 28 agents including new agents uncovered in the L1210 primary screen and older drugs of clinical interest (Table 1).

Pertinent Observations Included:

1. NSC-1895. Given IP, effectiveness increased as the interval between treatment decreased. Continuous treatment every 12 or 6 hours was more effective than daily treatment or treatment at wider intervals. Activity was reduced on oral administration.

2. NSC-8806. Maximum effectiveness was observed when agent was given every 3 hrs./24 hrs., every eighth day. On schedules tested, equivalent activity was observed using the IP, PO, and SC routes.

3. NSC-56054. Schedule dependency studies generally resulted in a bimodal distribution of deaths. Divided doses given over 24 hours on day 1 only or on days 1 and 9 provided the greatest number of long-term survivors.

4. NSC-82196. All schedules were very effective. Maximum activity appeared to result from a single treatment or intermittent treatment (every 4th or 8th day).

5. The following agents failed to exhibit marked schedule dependency: NSC-40774, 45388, 58404, 58514, 79037, 87974, 88106, 92188.

6. Seventeen agents were tested in the early L1210 system to determine the relative effectiveness of 8 divided doses or a single injection on day 1 only; days 1, 5, and 9; or days 1 and 9. The results indicated that NSC-1895, 8806, 56054, and 69945 were more effective when given in divided doses. In contrast, NSC-83265 and NSC-65346 were more effective when given as a single treatment.

The results of other schedule and route dependency studies and detailed results of the studies reported above are included in the Annual Progress Report, contract PH43-64-911.

II. Duration of Drug Levels of NSC-82196 - 5(or 4)-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole 4(or 5)-carboxamide

A method was developed which employs the survival time of leukemic mice to determine the extent and duration of the retention in the host of a given amount of NSC-82196 given SC, IP, or orally. With SC or IP administration, 50% of the administered dose was lost in the first 60-90 minutes; however, detectable activity persisted for 24 to 48 hours. The efficacy of this compound was greatly reduced when given orally. Because of the marked reduction in the therapeutic effect provided by oral administration of this agent, estimates of the percentage retention by this route were not feasible.

III. Development and Utilization of a Variant of L1210 Resistant to NSC-82196.

A leukemic L1210 variant (L1210/82196R) which is resistant to NSC-82196 was developed and tested for cross-resistance to a number of agents which are active against the parent tumor.

L1210/82196R was found to be cross-resistant to the following drugs:

NSC No.	Name
82196	Imidazole-4(or 5)-carboxamide) 5(or 4)-3[3,3-bis(2-chloroethyl)-1 triazeno]-
45388	Imidazole-4(or 5)-carboxamide, 5(or 4)-(dimethyltriazeno)-
87974	Urea, 3-cyclohexyl-1-(2-fluoroethyl)-1-nitroso-
88106	Urea, 1-(2-chloroethyl)-3-(2-norbornyl)-1-nitroso-
409962	1-nitrosourea, 1,3-bis (2-chloroethyl)-
79037	Urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-
406024	Urea, 1-methyl-1-nitroso-3-(1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinyl)-
23909	Urea, 1-methyl-1-nitroso-
47547	Urea, 1-(2-chloroethyl)-1-nitroso-
104800	Dibromodulcitol

L1210/82196R remained sensitive to the following drugs:

NSC No.	Name
26271	Cytoxan
740	Methotrexate
755	Purine-6-thiol, hydrate (6-mercaptopurine)
63878	Cytosine arabinoside
82151	Daunomycin
69945	Phosphoramide mustard
56410	Porfiromycin
1895	Guanazole
102816	5-azacytidine

IV. Sequential Chemotherapy with Cytoxan (NSC-26271) and Cytosine Arabinoside (Ara-C, NSC-63878) in Mice with Advanced Leukemia L1210

Studies with advanced stages of leukemia L1210 have demonstrated the advantage of therapy employing a single treatment of cytoxan, an agent capable of markedly reducing the leukemic population, followed by multiple treatments with ara-C. A priming dose of cytoxan followed by prolonged treatment with ara-C resulted in increases in survival time about 1½ times greater than that achieved by either drug alone. Increased toxicity was not evident when sequential therapy employed doses of ara-C which were optimal for treatment with that drug alone. The reduction of leukemic cells by cytoxan appeared to make the disease more responsive to sequential ara-C therapy. This study provides further support for the use of an effective priming agent to achieve more effective therapy with a second agent. Future studies are planned to investigate other potential priming and sequentially administered agents.

V. Combination Treatment with 6-Mercaptopurine (6-MP, NSC-755) and 6-Methylmercaptopurine riboside (NSC-40774) against Early Leukemia L1210 Inoculated IP or SC.

In mice inoculated IP with leukemia L1210, a therapeutic advantage was observed with early treatment of the disease when 6-MP was given daily in combination with NSC-40774. In contrast, however, no clear evidence of synergism was observed when these agents were administered in combination to mice inoculated SC with L1210 leukemic cells.

VI. Evaluation of New Agents in the L1210 Assay System

Two new analogs of cytoxan were compared with cytoxan for activity in the L1210 assay system. The data indicate that a single IP treatment on day 2 only with either of the 2 analogs, 1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)-amino]-3-(2-chloroethyl)tetrahydro-, 2-oxide (NSC-109,723) and 1,3,2-oxazaphosphorine, 3-(2-chloroethyl)-2-[(2-chloroethyl)amino] tetrahydro-, 2-oxide (NSC-109,724) was not superior to treatment with cytoxan. However, when the drugs were given weekly (a more effective schedule for cytoxan), NSC-109,723 and NSC-109,724 were clearly superior to cytoxan.

Three agents related to methylglyoxalbisguanylhydrazone (NSC-32946), 2-naphthoic acid, 4,4'-methylenebis[3-hydroxy-, compound with [(methylethanediylidene)dinitrilo]diguandine (1:1), dihydrate (NSC-96016); carbanilide, 4,4'-diacetyl-, bis(amidinohydrazone) (NSC-109,555); and carbanilide, 4,4'-diacetyl-, bis(amidinohydrazone), diHCl trihydrate (NSC-111,056) were clearly more effective than methyl-GAG. NSC-111,056 was effective against the methyl-GAG resistant variant of L1210, whereas NSC-96016 was ineffective.

1-Propanol,3,3'-(methylimino)di-,dimethanesulfonate,HCl (NSC-84641) and 1-propanol,3,3'-iminodi-,dimethanesulfonate, HCl (NSC-102,627) were found to be more effective than Myleran (NSC-750) and Dibromodulcitol (NSC-104,800). The superiority of NSC-84641 and NSC-102,627 over NSC-104,800 was found using intermittent treatment every 4th day; the most effective schedule for NSC-104,800. NSC-750, known to be inactive against L1210 when given daily, was found to be inactive on the intermittent treatment schedule.

VII. Biological Characterization Studies of the Lewis Lung Mouse Carcinoma (with Goldin, DeWys, and Vadlamudi)

As part of the CCNSC program for the development of assay systems utilizing experimental tumors with relatively long apparent doubling times (T_d), a comprehensive study of the growth kinetics and operating characteristics of the Lewis lung carcinoma was initiated in collaboration with Dr. A. Goldin and members of the Chemotherapy Research Division. Progressive measurements of tumor weight as a function of time indicated a T_d of approximately one day when the tumor weighed about 100 mg. However, tumors weighing 1-4 grams exhibited a T_d of approximately 4 days, and tumors weighing 4-7 grams exhibited a T_d of approximately 8 days. The intramuscular implant site appeared to be optimal for screening with this tumor. A method was developed for estimating tumor diameters thereby avoiding the sacrificing of the mice. Tumor-mouse strain specificity studies indicated that C57Bl/6 (the strain of origin) or BDF_1 hybrid mice would be suitable hosts for studies of drug activity against the Lewis lung tumor. Following implantation into the right hind leg, metastasis developed, primarily to the lungs. Employing Cytoxan (NSC-26271) as a standard agent, model systems suitable for screening against the tumor have been formulated.

Proposed Course:

Quantitative evaluation of drugs of potential clinical interest as outlined above under "General and Specific Objectives" will continue to be emphasized. Studies of schedule dependency, drug combinations, and the influence of therapy on the tumor cell population will be aimed at the ultimate objective; that is, the total eradication of the tumor. Studies of the kinetics associated with tumor cell proliferation will continue in connection with the establishment of new *in vivo* assays for drug evaluation. In this connection, increased attention will be accorded investigations of the operational characteristics of relatively slow-growing tumors and tumors which show a spectrum of drug sensitivity different from that of mouse leukemia L1210. Because of the need for increased primary screening capacity in the CCNSC program, it is anticipated that primary screening within this project will be initiated and conducted at a rate of approximately 10,000 tests per year.

Publications:

See current organization report, Office of the Chief, Drug Evaluation Branch, CCNSC.

Table 1. Agents Employed in Studies of Schedule and Route Dependency Against Early Leukemia L1210

NSC No.	Compound Name
1895	Guanazole
8806	L-Alanine,3-(*p*-[bis(2-chloroethyl)amino] phenyl)-hydrochloride
27640	Uridine, 2'-deoxy-5-fluoro-
40774	6-Methylmercaptopurine riboside
42076	Podophyllotoxin, 4,6-O-benzylidine-β-D-glucoside
45388	Imidazole-4(or 5)-carboxamide, 5-(or 4)-(dimethyltriazeno)-
56054	Pseudourea, 2,2'-(9,10-anthrylenedimethylene)bis-[2-thio-, dihydrochloride
56408	Tubercidin
56654	Azotomycin
57199	DL-Alanine, 3-(*o*-[bis(2-chloroethyl)amino] phenyl)-
58404	Glycine, N-(diazoacetyl)-, hydrazide
58514	Chromomycin A3
61487	Carbonic acid, bis(*p*-aminophenyl) ester, 2H Cl
65346	Sangivamycin
69945	Phosphoramide mustard
74841	Ethylenediamine, N-(2-chloroethyl)-, dihydrochloride
75520	Trifluoromethyldeoxy uridine (F_3TDR)
79037	1-Nitrosourea, 1-(2-chloroethyl)-3-cyclohexyl-
82151	Daunomycin

Table 1 (Continued)

NSC No.	Compound Name
82196	Imidazole carboxamide, bis-chloroethyl triazeno-
83265	3-tritylthio-L-alanine
85998	Streptozotocin
87206	Demethylellipticine
87974	1-Nitrosourea, 1-(2-fluoroethyl)-3-cyclohexyl-
88106	1-Nitrosourea, 1-(2-chloroethyl)-3-(2-norbornyl)-
92188	Isoquinaldehyde thiosemicarbazone
94100	Dibromomannitol
104800	Dibromodulcitol

BIOCHEMISTRY SECTION

DRUG EVALUATION BRANCH

Introduction

The objective of the Biochemistry Section is to explore and utilize biochemical approaches for improving the therapy of human cancer, whether by bringing about increased effectiveness of existing drugs, by increasing selective toxicity for cancer cells through the design of drugs to achieve particular biochemical effects, or by control of metabolic pathways. The program is implemented largely through contracts with universities and research institutes administered by Dr. Florence R. White, Section Head.

The inability of a drug to completely control human malignant disease can be a consequence of (1) natural or acquired resistance to the drug; (2) failure to use the drug in a way that will produce maximum antitumor specificity; or (3) failure to select the best drug for the particular patient's tumor.

When resistance to treatment develops, and can be related to a specific alteration in the metabolism of the tumor, modifications of the agent to fit the changed metabolism can be designed. For example, in some animal systems, resistance to thioguanine due to lack of pyrophosphorylase can be circumvented by giving the riboside, which is activated by a kinase, while the deoxyriboside will overcome a second mechanism of resistance due to loss of ability to convert ribosides to deoxyribosides.

One of the best clinical examples of improved therapeutic results due to changed mode of administration is methotrexate. The use of parenteral treatment on intermittent dosage schedules increased its effectiveness several fold. Sequential therapy with combinations of drugs may also give enhanced effects. However, study of both the mechanism of action of drugs, mechanisms of resistance, and an equally detailed metabolic characterization of the tumor may be necessary to plan which drugs to use in combination chemotherapy and how to use them, in relation to each other and to the tumor.

The third of the reasons for unsatisfactory drug therapy of cancer may be the result of too little fundamental knowledge of crucial metabolic aspects tumor growth and of drug action in relation to these aspects. An example of a possible approach to this problem is described in part 1 of the Major Findings presented later.

There are at present five program areas in which work is underway: (1) mechanisms of action of new and established drugs, mechanisms of resistance to chemotherapy, and means of overcoming resistance; (2) studies of dihydrofolate reductase; (3) evaluation of "masked" cytotoxic and cytoprotective agents; (4) synthesis and study of the biological effects of new types of purine and pyrimidine analogs; (5) alteration of certain antitumor agents by means of polymerization.

Some of these areas are closely related and overlap to a degree. Similarly, individual contracts often encompass effort in more than one area. This will be clarified as each program area is described.

Major Findings

(1) Mechanism of Drug Action, Mechanisms of Resistance to Chemotherapy, and Means of Overcoming Resistance. Work in this area is done as a major effort under contracts with Stanford Research Institute (PH43-65-575) and Southern Research Institute (PH43-66-29).

At Stanford Research Institute studies have been carried out on four new nucleosides and five other agents, three of which have been selected as candidates for clinical trial. The first of the new agents was 3'-deoxythioguanosine, which was an active antitumor agent against carcinoma 755, TA-3 carcinoma, and a thioguanine-resistant line of Ehrlich carcinoma. It was neither cleaved nor converted to nucleotide, and was rapidly excreted in the urine. It had little or no effect on de novo purine synthesis. However it inhibited the utilization of performed purines, particularly into the RNA guanine. Other new agents were 2'- and 3'-O-methylthioguanosine (NSC 105021 and NSC 106540 respectively). Both were active against L1210 leukemia; NSC 106540 at nontoxic high doses. NSC 105021 was particularly active in combination with azaserine. Using S-35 labeled drugs, the 3'-methylated thioguanosine was rapidly metabolized in mouse tissues. The metabolites, however, have not yet been identified. Both compounds were rapidly excreted in the urine. The last of the new compounds to be studied was the periodate oxidation product of 6-methylmercaptopurine riboside (NSC 111712). This compound caused 97% to 99% inhibition of Ehrlich ascitic cell production in vivo. It also caused 90% to 95% inhibition of tritiated-thymidine incorporation into DNA. This could be the result of inhibition of thymidine kinase, of thymidylate kinase, or of DNA polymerase. Study indicated that the inhibition was only on thymidylate kinase, which catalyzes the conversion of thymidine monophosphate to thymidine triphosphate.

Studies on the mechanism of action of adenine arabinoside and adenine xyloside continued. Both compounds were active as triphosphates and were substrate for adenosine deaminase, with the corresponding inosine derivatives inactive. The biochemical effect of adenine arabinoside related to antitumor activity was noncompetitive inhibition of DNA polymerase. That of xylosyl adenine was inhibition of phosphoribosyl-pyrophosphate synthesis.

Since mouse blood was high in adenosine deaminase, as were many mouse tumor cells, selection of a test system for these compounds presented a problem. Ridgeway osteogenic sarcoma and carcinoma-1025 were low in the enzyme, but, since they were mouse tumors, the drugs must be used with an adenosine deaminase inhibitor to obtain a response. This has not yet been accomplished. The Dunning rat leukemia was relatively low in deaminase activity as was the blood of the Fisher rat. However, tests with this tumor system according to CCNSC protocol, did not show activity. Since arabinosyl- and xylosyl-adenine must be converted to triphosphates for activation, it is possible that rat tissues were unable to carry out activation at an adequate level.

Human tissue biopsy specimens have been assayed for activity of nucleotide formation from the α- and β-analogs of 2'-deoxythioguanosine (NSC 71851 and NSC 71261 respectively). Similar to previous findings with frozen tumor specimens, the fresh biopsy material showed considerable variation in ability to activate these two anomers, between histologically similar tumors from one patient and another. This indicates that it should be possible to select patients whose tumors would respond to one or both of these agents. Different portions of the same tumor were also analyzed separately. There were appreciable variations, as would be expected in heterogeneous tumors, but the differences were probably not great enough to change a conclusion as to whether or not a tumor would be responsive.

At Southern Research Institute studies continued on the mechanism of action of 6-methylmercaptopurine riboside (6MeMPR; NSC 40774). This drug has been found to be the most potent inhibitor among many nucleosides, of phosphoribosylamido transferase, catalyzing the production of phosphoribosylamine from glutamine and phosphoribosylpyrophosphate. It has been found that IMP, MPRP and MeMPRP were bound at the same site, while $6NH_2$- and 6OH-purines were bound at a different site on this enzyme. Hence, the potentiation of MP and MeMPR against experimental tumors was not due to effects on this enzyme. The mechanism of this potentiation is being sought, as are other potentiating combinations, such as 6-thioguanine and 6MeMPR.

Continued study of the mechanism of action of alkylating agents _in vivo_ indicated that their action is not due to direct interference with DNA polymerase.

Other agents whose mechanisms of action were under study were: carbocyclic adenosine (NSC 103526) in which $-CH_2-$ replaces the -O- of the furane ring of ribose, guanazole (NSC 1895), and 8-azainosine. The latter has long been known as a cytotoxic agent. Labeled drug is now being prepared to study its mechanism of action.

Guanazole, another known agent recently found active in L1210 leukemia, was found to inhibit DNA synthesis but not that of RNA or protein. Inhibition of DNA synthesis was not by an effect on DNA polymerase. L1210 cells exposed to guanazole _in vitro_ showed decreased viability upon _in vivo_ implantation.

Carbocyclic adenosine was an active antitumor agent. It was phosphorylated and deaminated but not incorporated into nucleic acid or NAD-analogs. It did inhibit _de novo_ synthesis of purines and could act at an as yet unidentified block on the metabolism of free purine bases or nucleotide interconversions.

Complete cell cycle times as well as estimations of the proliferating fractions were completed for L1210 leukemia _in vitro_ and _in vivo_, hamster plasmacytoma, sarcoma-180, carcinoma-755 and dimethylbenzanthracene (DMBA) induced tumors. The proliferating fraction was always less than 100%, and varied from 85% for sarcoma-180 to 3%-5% for DMBA-induced tumors. This, and biological evidence, indicate that some of the tumor cells exist in a G_0, or indefinitely prolonged G_1 phase, in which they are viable but non-proliferating.

The effects of fourteen agents upon the cell cycle have been studied. These were: cytosine arabinoside (NSC 63878), fluorodeoxyuridine (NSC 27640), hydroxyurea (NSC 32065), methotrexate (NSC 740), 6-mercaptopurine (NSC 755), methylmercaptopurine riboside (NSC 40774), nitrogen mustard (NSC 762), puromycin (NSC 3055), actinomycin D (NSC 3053), azaserine (NSC 742), mitomycin C (NSC 26980), thio-TEPA (NSC 6396), trimethylmelamine (NSC 9706), and 1,3-bis (2-chloroethyl)-1-nitrosourea (NSC 409962).

(2) Study of Dihydrofolate Reductase. Work in this area is being done at Scripps Clinic and Research Foundation under contract PH43-65-14 and at Yale University School of Medicine under contract PH43-67-1193.

Dihydrofolic reductase is considered to be the specific target of methotrexate. Thus knowledge of whether the enzyme is identical in normal and malignant tissues, in mouse and man, in sensitive and resistant tumors, whether there are isoenzymes, and the nature of the active site(s) should be useful in achieving the maximum therapeutic effect with methotrexate itself and in the possible design of desirable new drugs.

During the past year it has been found at Scripps that purified chicken liver enzyme, subjected to electrophoresis, separated into three distinct bands. That these forms were stable, rather than equilibrium products, was shown by subjecting isolates of each of the two most abundant forms to further electrophoresis. In each case the material moved in a single band. Further studies of isolated material from bands I and II showed them to be identical with respect to: molecular weight (22,000), the ratio of activity on folic acid to dihydrofolic acid, a double optimum pH (4.5 and 7.5), activation by mercurials, lack of activation by ions, and sensitivity to methotrexate. At this time electrophoretic motility is the only observed difference between these enzymes. Dihydrofolate reductase from L1210 cells shows the three band pattern. The electrophoretic pattern of dihydrofolic reductase from human chronic myelogenous leukemia cells will be studied when it is sufficiently purified and the concentrated enzyme is obtained.

Studies to determine, at the molecular level, the manner in which folate antagonists are bound to dihydrofolate reductase is being approached in two ways: (a) by noting the effect of structural variations in the inhibitors on their inhibitory power, and (b) by obtaining information on the nature of the inhibitor and substrate sites of the enzyme. Chemical changes in amino acids known to be at, or near, these active sites were related to loss of catalytic activity and/or loss of ability to bind substrates. Oxidation of the thiol group of cysteinyl residues, possibly to sulfinic acid, lead to activation. When destruction of the tryptophan residues by N-bromosuccinimide, which selectively cleaves the indole ring oxidatively, reached 1.05 of the four residues known to be present, there was complete loss of activity. Graded amounts of fluorodinitrobenzene, known to destroy lysine, will be the means to follow the effects on enzyme activity of loss of progressive numbers of its 16 lysine residues.

At Yale University agents which may be useful in human therapy are selected with the use of 10 mg. or less of agent. Compounds, most of which are supplied by CCNSC, are assayed for their ability to inhibit dihydrofolate reductase from human sources. The extent of degradation by human liver aldehyde oxidase of those compounds showing a useful level of antifol activity is then determined.

(3) Evaluation of "Masked" Cytotoxic and Cytoprotective Agents. Work in this area is underway in part of the contract with Southern Research Institute, PH43-66-29.

Substrates consisting of cytotoxic or cytoprotective agents "masked" chemically in various ways have been synthesized by Collaborative Research, Inc. If the masking group is removed *in vivo*, either nor-nitrogen or sulfur mustard as cytotoxic agents, or cysteamine or cysteine as x-ray or mustard protecting agents, will be released at the site of cleavage or activation. If there is a difference in activating enzyme activity between tumors and critical normal tissues, therapeutic selectivity should be possible.

Evaluation of a representative group of such agents was done at Southern Research Institute. Background information on the uptake of tritiated thymidine in a spectrum of hamster tissues and in alkylating-agent sensitive plasmacytoma was established. The effect of the "masked" cytotoxic agent on the spectrum of tissues was determined. None of the agents showed any selective activity, and some were non-toxic, which showed that the "mask" was not biologically removable in the hamster.

(4) Synthesis and Study of the Biological Effects of New Types of Purine and Pyrimidine Analogs. Work in this area is being done at the University of Connecticut under contract PH43-65-1035.

The objective of this contract has been to synthesize some pyrimidine and purine derivatives which may have selective cytotoxic effects. The principal series of agents are to be trinucleoside phosphates in which three natural or analog bases are combined with ribose or other sugars which are attached to a single phosphate. This will yield an electrophoretically neutral molecule which should be taken into cells. By this means it may be possible to introduce bases which are not normally transported into cells, as well as to by-pass some types of drug resistance.

The trinucleoside phosphate of di-deoxyuridine-6-mercaptopurineribonucleotide, labeled with P^{32} and S^{35}, is being prepared in order to determine whether or not compounds of this kind are taken into cells intact or are split at the cell surface. Incubation of mammalian cells with the doubly labeled compound, followed by identification of intracellular labeled materials by paper or thin-layer chromotography should give indications of the utility of nucleosides of this class.

(5) The Alteration of Certain Antitumor Agents by Means of Polymerization. Work in this area has started at the Weizmann Institute of Science, Rehovoth, Israel under Foreign Research Agreement (PL-480) No. 635125.

The work coming from Dr. Ephraim Katchalski's laboratory has indicated that polymers of amino acids may have physical properties of biological importance, which may be controlled by the nature of the amino acids making up the polymer.

For example, when enzymes are "coated" with such polymers they retain their activity, but, due to the polymer, are independent of fluctuating environomental changes such as pH etc. Because many antitumor agents contain groups which trigger polymerization, it seemed possible that the proper polymer coating of an active drug could give the drug selectivity which might have considerable usefulness.

Work over the past year included the synthesis of oligopeptides of alanine, ornithine, lysine, histidine, and a copolymer of ornthine and leucine. Utilization of some of these peptides was studied by following the synthesis of induced β-galactosidase in suitable mutant strains of E. coli. While mono-, di-, and tri- lysine and ornithine were readily utilized, higher homologues were not. In the wild type of E. coli, tri- and tetra- lysine as well as tri-, tetra- and penta-ornithine inhibited galactosidase synthesis when added to the media at the same time as the inducer.

Spermidine and putrascine, which are normal constituents of the "walls" of both E. coli and phage, reversibly inhibited phage synthesis. The inhibition was of DNA synthesis and resulted in lack of "late" enzyme production. "Early" enzyme formation was unaffected.

Proposed Future Course

Plans for future programs through which the overall goals of the Biochemistry Section may be approached, should funds permit, are:

(1) To investigate and utilize biochemical tests to provide a rapid means of determining likely biochemical sites of drug action. Such studies will be aimed at making a first approximation regarding the particular site of inhibition leading to selective toxicity against tumor cells and employing such information for helping in the design of biological experiments to determine the optimal treatment schedule for maximum tumor cell destruction or alteration in the kinetics of cell regeneration.

(2) To study enzyme distribution within and among tissues and the effects of drugs on them by histochemical means.

(3) To study cell membranes of normal and malignant tissues.

(4) To investigate the control of protein synthesis through a study of factors which may influence rate of mRNA production and its transcription.

(5) To search for possible deletions of allosteric control of enzymes in cancer.

IMMUNOTHERAPY AND IMMUNE RESPONSE MODIFIERS SECTION
DRUG EVALUATION BRANCH

The major activities of this section have been concerned with studies of tumor associated antigens and host reactivity to such antigens with a view to advantageously utilizing the knowledge acquired from such studies for the development of immunotherapeutic models for the treatment of mouse neoplasms. This work was performed by a contractual arrangement with Microbiological Associates, Inc. at the Kenwood Laboratories where NCI staff and employees of the company participate in a collaborative effort. The accomplishments under this arrangement are described in a separate report (See this report, Serial Number 3577).

The Head of this Section, as project officer and member of the Special Animal Ecology Work Group of the Special Virus Leukemia program, administered five contracts. At the Albert Einstein Medical Center in New York (PH43-65-612), Dr. Frank Lilly is studying the genetics of susceptibility to virus-induced leukemogenesis. Dr. Robert Marshak of the University of Pennsylvania School of Veterinary Medicine (PH43-65-1013) is investigating the etiology of bovine lymphosarcoma. At the State University of New York, Dr. Charles L. Burger (PH43-65-605) is developing separative procedures for the isolation, purification and concentration of candidate human and bovine agents. Dr. Charles Rickard of the School of Veterinary Medicine, Cornell University (PH43-65-620) is studying the etiology of feline leukemia. Dr. M. Michael Sigel of the University of Miami (PH43-67-1187) is studying procedures for preparing an effective vaccine against avian leukosis.

Serial No. NCI 3577
1. Chemotherapy, CCNSC
2. Drug Evaluation Branch
3. Immunotherapy and Immune Response
   Modifiers Section
4. Wiscon Building - Kenwood Laboratories
   Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Transplantation and Serological Studies with Virus-induced Murine Leukemias and their application to therapy.

Previous Serial No.: Same

Principal Investigator: J. P. Glynn

Other Investigators: A. Fefer

Cooperating Units: None

Man Years

Total: 3
Professional: 2
Other: 1

Project Description:

Objectives:

The investigation of tumor associated antigens and the host reactivity to such antigens with a view to utilizing the knowledge of both for the development of immunotherapeutic models for mouse leukemia.

Method Employed: Standard laboratory procedures were employed.

Major Findings:

The antigenicity of murine leukemias induced by Friend, Moloney and Rauscher (FMR) viruses was investigated by extensive transplantation and serologic studies. Results obtained by the indirect fluorescent antibody technique, the antibody absorption test and the transplantation tests are consistent with the view that tumors induced by the three viruses possess common antigens. Whether the relevant antigens are virion or cellular is unknown, however it was recently demonstrated in complete cross-neutralization tests, employing FMR viruses and specific anti-FMR sera, that the oncogenicity of all three FMR viruses was effectively neutralized by each anti-FMR serum. These results suggest that Friend, Moloney and Rauscher viruses possess similar, if not identical antigens.

The presence of tumor associated antigens permits the immunization of mice against the transplantation of histocompatible tumor cells. Immunization is readily achieved by pretreating with infectious virus or with syngeneic or allogeneic tumor cells containing infectious virus. Preparations of viruses, rendered non-infectious by treatment with formalin, are incapable of inducing transplantation resistance despite their ability to induce the formation of anti-viral antibody.

Immunization, however, is not only a function of the immunogen, but is also dependent upon the immunologic reactivity of the host. Comparative studies on transplantation resistance in BALB/c and C57BL/6 mice in the FMR tumor systems have demonstrated that transplantation is readily induced in C57BL/6 mice, whereas, it is induced only sporadically in BALB/c mice. This difference is not a function of the intrinsic immunogenicity of the FMR tumors originating the two strains, because tumors of both C57BL/6 and BALB/c origin were immunogenic in C57BL/6 mice. The difference in the case of induction of transplantation resistance reflects a difference in host reactivity. Indeed, transplantation resistance in BALB/c mice was more readily induced by pretreatment with tumors releasing the least amounts of infectious virus. Therefore, there appears to be an inverse relationship between the susceptibility to viremia and the susceptibility to the induction of transplantation resistance.

Knowledge derived from extensive experiments with a new and unique tumor model, a virus-induced murine sarcoma, is consistent with the above formulation. Moloney sarcoma virus (MSV) rapidly induced local tumors in newborn BALB/c, C57BL/6 and CBF mice. However, almost all tumors induced in BALB/c mice grew progressively, 40% of tumors induced in C57BL/6 mice and 24% of the tumors induced in CBF mice completely regressed. Sera from mice whose tumors regressed neutralized the oncogenicity of MSV. Adult mice of the three strains were equally susceptible to oncogenesis by MSV. Temporary immunodepression of the host by X-irradiation did not affect susceptibility to tumor formation. However, all tumors developing in normal adult mice regressed, only 50% of induced tumors in irradiated mice regressed. The findings suggest that in this tumor system, the immunologic competence of the host is not a critical factor in its susceptibility to oncogenesis, but may determine whether a tumor once formed, will regress or kill the host.

Tumors induced by MSV were shown to possess tumor associated antigens. Transplantation and indirect fluorescent antibody studies showed that these antigens are common to tumors induced by FMR viruses. In addition, it was demonstrated that serum from animals immunized with FMR or Gross lymphomas were capable of neutralizing the oncogenicity of MSV.

The mechanism of tumor rejection in the MSV system was studied by an _in vitro_ cell neutralization test. Incubation of Moloney sarcoma cells with lymphoid cells from histocompatible mice specifically sensitized to MSV prevented their outgrowth in X-irradiated immunologically depressed histocompatible mice. This finding is consistent with the hypothesis that tumor rejection is largely mediated by immune lymphoid cells.

In order to investigate this problem further, adult mice whose autochthonous MSV-induced tumors ultimately regressed, were studied, to determine; 1. The development of long-term resistance to MSV reinoculation; 2. The production of virus-neutralizing antibody and the development of resistance to transplanted syngeneic Moloney sarcoma cells, as a function of autochthonous tumor growth and regression; 3. The MSV content of autochthonous tumors, as a function of their growth and regression; 4. The pathology of regressing tumors.

A summary of the data reveal that tumors induced in immunologically unresponsive hosts grew progressively contained abundant virus, were not infiltrated with lymphoid cells, and did not induce the production of detectable quantities of virus-neutralizing antibody. By contrast, almost all tumors induced in normal adult mice ultimately regressed. The regression was associated with a progressive increase in infiltration with lymphoid cells and mononuclear cells and a decrease in virus content, and was preceded, accompanied, and followed by the production of virus-neutralizing antibody and resistance to the transplantation of syngeneic Moloney sarcoma cells.

The demonstration that autochthonous tumor regression occurs with the MSV-induced tumors in mice increases the number of species to three, (chickens, mice and rabbits) in which this phenomenon has been described. This observation lends strength to the possibility that an immunologically mediated rejection of grossly undetectable microscopic nest of antigenic tumor cells by autochthonous hosts may be occurring in other tumor systems and even in nature.

Knowledge derived from studies of tumor antigens and immunological reactivity of mice to viruses and their induced tumors permits construction of an immunotherapeutic model. A rather simplified chemo-immunotherapeutic model has been developed with a leukemia induced by Moloney virus in BALB/c mice. Mice were inoculated with a histocompatible Moloney lymphoma. Approximately one week later, when tumors were locally palpable and had spread systemically, the mice were injected with cytoxan and spleen cells from F_1 hybrid mice specifically sensitized to the Moloney antigen(s). The impetus for the series of experiments came from several sources: 1. Although conventional chemotherapy of MV leukemia occasionally provided marked prolongation of survival time, the treated animals eventually died of leukemia; 2. Since MV-induced leukemias, regardless of the strain of origin possess similar or related transplantation antigens, immunization procedures could be employed which would preclude any positive effects being due to normal murine isoantigens; 3. Cyclophosphamide at dosage levels therapeutically effective against MV-induced leukemias, has been shown in conjunction with F_1 hybrid spleen cells to induce chimerism in BALB/c mice; and 4. The availability of specific allotypic sera provided a convenient method for the serial determination of the persistence of the chimeric state. Briefly, the results were as follows:

1. Untreated tumor bearing mice survived for about 10 days.

2. Tumor bearing mice inoculated with normal or immune spleen cells survived a comparable period of time.

3. Tumor bearing mice inoculated with optimal doses of cytoxan with or without normal spleen cells survived for 20-25 days.

4. Tumor bearing mice inoculated with optimal doses of cytoxan and immunized spleen cells became completely tumor free and approximately 50% of them survived for 200 days and 25% survived for approximately 2 years. Donor gamma-allotype generally persisted during this period, no animal with persistent allotype developed leukemia.

5. Two additional drugs, BCNU and cytosine arabinoside, tested for their ability, at therapeutic doses, to induce chimerism were ineffective.

6. BCNU, which is as therapeutically active as cytoxan against the tumor in the above model was ineffective in the immunotherapy model.

Significance:

The purpose of these studies is to obtain a better understanding of the nature and biology of tumor antigens and the specific immunological responses elicited in the tumor-bearing host. This knowledge can be applied to model systems in mice involving combination immuno-chemical therapy, which may lay the framework for subsequent clinical studies.

Proposed Course:

During the coming year, a major effort will be made with immunotherapy models similar to the one described in the current report. Strong emphasis will be placed on studies concerned with the role of tolerance in the development of primary neoplasms.

Publications:

See current organization report of Drug Evaluation Branch.

SCREENING SECTION

DRUG EVALUATION BRANCH

Objectives and Significance to the National Cancer Institute

The principal objectives of the Screening Section, Drug Evaluation Branch, (DEB) are the selection of new potential candidate drugs for clinical trial against cancer and the investigation of means for increasing the therapeutic usefulness of older drugs. To achieve these objectives the Screening Section coordinates a nation-wide program, largely through contractual arrangements with qualified institutions, for (a) screening of a large number of synthetic and natural products in *in vivo* and to a limited degree *in vitro* assay systems, (b) biological assay required for directing fractionation of natural products for the isolation of the active component, (c) detailed evaluation and development of materials which emerge from primary screens including investigation of factors that influence therapeutic efficacy, (d) presentation of experimental results pertinent to the selection of new agents for clinical trial, to the Chemotherapy Program, NCI and (e) methods research for the development of new and improved assay systems which may predict new clinically useful compounds.

Drug activity against experimental tumors *in vivo* is the principal basis for selecting candidate drugs for clinical trial and for furnishing leads relative to optimal conditions for drug usage. *In vitro* screening against neoplastic cells or microorganisms provides an effective method for recognizing biologically active properties in materials submitted in insufficient quantity for *in vivo* screening, for following fractionation and isolation of active components from crude natural products with demonstrated activity both *in vivo* and *in vitro*, and for detection of agents that might be useful against infectious organisms which may complicate clinical treatment of malignant disease.

The Screening Section supervises the work of contract laboratories; coordinates the total effort with other projects within the DEB, the CCNSC, and the Chemotherapy Program, NCI; determines and designs assay systems to be used and criteria for drug activity; investigates usefulness and feasibility of new assay systems designed to enhance predictive value for the clinic; recommends candidate drugs for clinical trial to the Chief, Drug Evaluation Branch, Associate Scientific Director and Chief, CCNSC, and to the Scientific Director for Chemotherapy. Prepares data for review by the Chemotherapy Staff. The staff provides pertinent information to the Drug Evaluation Committee, Operating Committee, Experimental Therapeutics area and Clinical Trials area of the Chemotherapy Program. Thus, the Screening Section represents a major part of a well integrated program devoted to the evaluation of more useful antitumor chemotherapy and to the intensive investigation of new agents.

The present report is divided as follows:

I. Staff Functions

II. Significant Accomplishments

A. Primary Screening

B. Selection of Candidate Drugs for Clinical Trial

C. Studies of New and Old Drugs of Clinical Interest

D. Methodology (Research and Development)

III. Related Service Projects

IV. Proposed Course of Projects

I. Staff Functions

The Screening Section is staffed by seven professionals and three statistical and clerical personnel. The principal investigators are: Betty J. Abbott, Head, Screening Section, David Abraham, (CO), Mario Baratta (CO), Ruth I. Geran, and Mary M. Macdonald. In carrying out its responsibilities staff members (1) review proposals for institution or renewal of contracts, recommend new projects to the Chief, Drug Evaluation Branch, and prepares associated materials for NCI program and contract reviewing bodies; (2) serve as Project Officers for contract projects, provide guidance and instruction, advise on scientific and technical sufficiency, and indicate areas requiring modification or clarification through day to day telephone and mail communication and project site visits; (3) establish priorities for testing and instruct laboratories in the conduct of experiments of special interest; (4) recommend and design new assays for screening and detailed drug evaluation, define parameters of response, establish criteria for evaluation and direct laboratory testing; (5) advise the Program Analysis Branch, Chemotherapy Program, of needs relative to data retrieval and collaborate on the establishment of appropriate procedures; (6) advise the Mammalian Genetics and Animal Production Section, CCNSC, of animal needs for screening, animal disease problems and design experiments to evaluate animal strains; (7) organize, review, and summarize experimental results; (8) instruct screeners regarding needed additional data; (9) advise natural product chemistry laboratories under contract to the Natural Products Section, Drug Development Branch, of crude materials which are worthy of fractionation and isolation studies; (10) assign agents of potential clinical trial to detailed studies for the determination of optimal conditions for drug usage; (11) determine activity in the primary screen and collectively as members of the Drug Evaluation Committee recommend new agents as candidates for clinical trial.

II. Significant Accomplishments

Some important accomplishments of the Screening Section during the calendar year of 1967 are listed below. These and other contributions to the Chemotherapy Program are discussed more fully in parts A through D of this section.

(1) Improvement of experimental designs for the detailed evaluation of active agents particularly with respect to providing information on treatment schedule and route dependency.

(2) Selection, through the Drug Evaluation Committee, of 14 materials as potential candidate drugs for clinical trial.

(3) Continued development and use of experimental designs for determining the ability of drugs to destroy tumor cells or to otherwise influence the kinetics of tumor cell proliferation.

(4) Continued development of antimicrobial screens against various bacteria and fungi present as fulminating infectious in terminal cancer patients.

(5) Initiation of methodology studies for the development of four potential new tumor systems for the screening of materials which may inhibit relatively slow growing tumors.

(6) In collaboration with the Program Analysis Branch (see current annual report)(PRA) provided day-to-day advice relative to the updating of the mechanism for the computer processing of data in order to provide increased efficiency in data evaluation.

A. Primary Screening

Primary screening consists of the original testing of synthetic, plant fermentation and animal products submitted to the CCNSC program. Testing is conducted under protocols designed to select initially a modest number of materials which are then subjected to progressively exacting requirements which are designed to select those having the greatest antitumor potential. Based upon known correlations between laboratory experimental results and clinical effects primary screening of new material has been conducted during the past year against mouse leukemia L1210 (LE) and against the Walker rat intramuscular carcinosarcoma 256 (WM) although the latter system was deemphasized during the year. The overall screen involved three phases of testing: (1) Treatment of mice with L1210 over a number of dosage levels on Day 2 only, (2) Daily treatment of mice with L1210 and (3) Treatment of rats with Walker 256 (WM). The general protocol for primary screening was presented in the Screening Section Annual Report for FY 1967. Modifications in the protocol are made when indicated. If an agent shows marked activity in phase one (single treatment LE dose response), it is removed from the routine screen and priority is given to its detailed evaluation in the LE system. In addition to its apparent high degree of predictability for clinical usefulness, (Table I), this screening plan resulted in a discernible shortening of the time required to receive initial screening results and a substantial increase in the percentage of submitted materials that were screened _in vivo_. For example, the screen permits phase one testing with only 150 mg. of a synthetic chemical of unknown toxicity. Previous to the institution of the three-phase screen, all _in vivo_ screening involved chronic treatment, and synthetic materials received in amounts of less than 500 mg. were tested _in vitro_ only. The number of materials now tested _in vivo_ is reflected in the analysis of the distribution of synthetic compounds shown in Table 2.

Testing continues to be carried out in alternative systems for special purposes; for example, (1) evaluation of selected agents against the LPC_1

plasma cell tumor, a test system which appears to mimic the clinical performance of multiple myeloma (see current individual project report, NCI Serial No. 3578), (2) evaluation of apparent structural analogs of known compounds for cross resistance against drug resistant tumor variants, (3) specific structure-activity studies, (4) evaluation in broad tumor spectrum of drugs emerging from other programs and drugs of demonstrated clinical usefulness which have been inactive in LE or WM.

During calendar year 1967, 7,413 new synthetics, 6,253 new crude plant extracts, 4,982 new crude fermentation products, and 446 new crude animal products were submitted to the screening program. The bulk of the testing in the presumptive screening is carried out by four laboratories: Battelle Memorial Institute (PH43-62-495), Hazleton Laboratories (PH43-63-622) and Illinois Institute of Technology (SA-43-ph-4349), and Wisconsin Alumni Research Foundation (PH43-66-494).

In addition to those few synthetics received in quantities too small for _in vivo_ testing and which were tested _in vitro_, (Table 2), there was continued utilization of cell culture for the follow-up assay of fractionation for plant products where a correlation with _in vivo_ activity _in vitro_ testing is carried out at the University of Miami (PH43-64-890), Arthur D. Little, Inc. (PH-43-65-61) and the University of Wisconsin (PH-43-67-1126).

B. Selection of Candidate Drugs for Clinical Trial

Those agents demonstrating reproducible activity in the primary screen (designated as Step 3 agents) are reviewed by the Drug Evaluation Committee which is comprised of the Chief, Drug Evaluation Branch, (Chairman); the professional staff of the Screening Section, and representatives of the Drug Development and Program Analysis Branches. The Drug Evaluation Committee reviews existing data and selects initially as potential clinical candidates (Step 4 agents) those which fulfill minimum requirements with respect to degree of activity. Further selection by the Drug Evaluation Committee is based upon molecular structure and quantitative or qualitative aspects of activity and the total evaluation is designed to answer specific questions of pertinence in considering the clinical potential of the agent. Materials of apparent clinical promise are recommended to the Chief, CCNSC, and the staff of the Chemotherapy Program as candidates (Step 4A) for preclinical toxicology and clinical trial.

C. Studies of New and Old Drugs of Clinical Interest

In addition to the review of individual compounds by all members of the Screening Section staff in connection with the Drug Evaluation Committee two mechanisms exist for the careful monitoring of drugs of both potential and known clinical interest. This is achieved through the Selected Agents Unit (SAU) composed of three professional and two statistical and clerical personnel of the Screening Section staff and the Screening Committee which has as original members four of the professional staff of the Screening Section

It is the responsibility of the SAU to: (1) recognize and segregate compounds with demonstrated activity in the primary screen, (2) determine adequacy of initial screen and confirmation, (3) schedule additional screening for inadequacies which exist and, (4) evaluate data and prepare summary charts and tables on Step 3 agents for the Drug Evaluation Committee and other reviewing bodies. The SAU maintains comprehensive records on antitumor activity for all agents acquiring Step 3 status in the primary screen and on other agents of special interest to the chemotherapy program staff. The SAU and the Screening Committee provide the principal liaison between the Screening Section, DEB, CCNSC and the Pharmacology and Toxicology Unit, Laboratory of Chemical Pharmacology, Experimental Therapeutics.

Table 3 shows the status of agents followed by the SAU or by the Screening Committee. During calendar year 1967, 201 agents displayed confirmed activity in the primary screen and were assigned to Step 3. During this period these and previously screened Step 3 agents comprised the 399 compounds reviewed by the Drug Evaluation Committee. Of the 230 materials reviewed which met Step 4 requirements 14 were accepted as candidates for clinical trial, (Table 4); while 213 were assigned to Step 4B. Step 4B compounds are those that exhibit sufficient activity to be considered for further development but which are recognized as structural congeners of known active agents and which did not elicit superior activity or failed to inhibit tumor variants selected for resistance to the known agent. A total 88 compounds were deferred after more detailed testing indicated insufficient activity to warrant further interest. Detailed or secondary evaluation includes studies which are supplemental to the establishment of activity in the primary screen. Such studies are conducted on Step 4A compounds primarily, but Step 4B compounds and compounds of interest emerging from other programs are often studied in detail. Drugs which have been in clinical trial are studied further because of clinical findings in recent years which indicate that older drugs can display enhanced therapeutic effects when used under more advantageous conditions of treatment schedule and route. Agents of clinical interest or potential clinical interest are subjected to intensive studies designed to determine the extent to which modifications in drug usages particularly with respect to route and schedule might increase therapeutic usefulness. Compounds are initially scheduled for schedule dependency studies according to the protocol detailed in Table 5. During calendar year 1967, 70 compounds were scheduled for treatment schedule and/or route studies (Table 6). Particular attention is given to determination of activity by the oral route as an indication of extent of gastro-intestinal absorption. With respect to the treatment schedule, emphasis is centered on providing leads for the clinic regarding the effect on therapeutic efficacy of the interval between treatments, the total number of treatments, and the total duration of treatments. In order to assure careful scheduling and appropriate follow-up of such studies the Screening Committee was instituted in July of 1967. Since this time, 32 compounds have been carefully reviewed by this committee. Follow-up testing scheduled were appropriate and the results reported to the Chief, DEB, and to appropriate scientific staff members.

Additional studies related to secondary evaluation are emphasized for those materials which are in early Phase I clinical trial and those which are regarded as the most likely candidates for early clinical trial. Such studies, in addition to investigations of the optimal conditions for treatment, include investigations of (a) influence of the tumor site on drug effectiveness (e.g., effects against intracranially inoculated tumors as an indication of the extent of the "blood brain barrier" to therapy, (b) effects of concomitant or sequential combination chemotherapy, (c) effect of new therapy on the development of resistance or on overcoming resistance to treatment, and (d) mechanisms of drug action. Such studies are conducted to provide information relative to the problem of the total eradication of experimental malignant disease.

The detailed evaluation of active drugs is conducted to some extent in all contract testing laboratories, but the major efforts in this area as well as in the closely related research and development area ("Methodology") are concentrated at the Southern Research Institute (PH43-65-594), Arthur D. Little, (PH43-65-61) and Microbiological Associates, Inc. (PH43-64-911). Specific studies conducted by, or in collaboration with, these institutions are described in the current report, Office of the Chief, DEB, the current individual project reports of the Branch's staff (NCI Serial Numbers 3576, 3577, 3578), and the Annual Progress Reports of the contracting laboratories.

D. Methodology (Research and Development)

Research and development consists primarily of studies involving investigations of experimental designs which may lead to more useful methods for drug evaluation and to selection of drugs with greater clinical efficacy. During the last year emphasis was again placed on the establishment of experimental protocols for screening and secondary drug evaluation consistant with available knowledge of: (1) correlations between clinical effectiveness and activity in laboratory systems and, (2) the kinetics of tumor and normal cell proliferation and the relative effects of drugs thereon. The following projects were among those conducted to provide information relative to these questions.

1. Development of a Walker 256 Survival System (WI). In order to acquire treatment schedule data for agents displaying activity in the WM system in which drug evaluation is based on the weight of the intramuscular tumor and the therapeutic index, the group at Southern Research Institute (PH43-65-594) has carried out the necessary developmental work to establish a Walker system in which ascitic Walker cells are implanted intraperitoneally. Drugs are evaluated for their ability to increase survival time of the rats. This system which appears to be both reproducible and selective has provided important information on drugs of clinical interest.

2. Methodology Related to Antitumor Specificity of Drug Action. Work in this area has centered around the growth kinetics of L1210 Leukemia and other experimental tumors. Continued studies at Arthur D. Little, Inc. (PH43-65-61) leading to the establishment of assays for assessing relative sensitivities to drugs of Leukemia (L1210) and normal bone marrow hemopoietic cells. This assay is based upon colony formation in the spleens of recipient animals following IV inoculation with normal or leukemic cells. The value of this system has been demonstrated previously and the evaluation of additional compounds is currently being carried out. The spleen colony assay has been

extended to include the P388 mouse leukemia. The group at Southern Research Institute (PH43-65-594) have continued their studies related to the influence of drugs on the kinetics of tumor and normal cell proliferation. Therapeutic efficacy is based on the percentage of tumor cells destroyed. Studies of drug effects on L1210 cells have been extended to other transplantable tumors and to spontaneous tumors including the C_3H mammary tumor and AKR leukemia.

Because of the increase of concern over the need for drugs which would be beneficial to solid tumor therapy 4 additional tumor systems are currently under intensive study in order to ascertain whether they might select drugs useful in this area. These systems, all of which represent tumors believe to have relatively long doubling times, were assigned to laboratories for intensive developmental studies in December of 1967 as follows: (1) Lewis lung carcinoma, under study at Microbiological Associates, Inc. (PH43-64-911), (2) Hepatoma 129, under study at Charles Pfizer and Company (PH43-68-45), (3) B16 Melanoma, under study at Arthur D. Little, Inc. (PH43-65-61), (4) the Harding-Passey Melanoma, under study at Southern Research Institute (PH43-65-594). It is anticipated that after studies are completed at least one of these systems will be incorporated into the primary screen for use as a routine testing tumor.

The program at Southern Research Institute for finding agents of potential use in controlling fulminating terminal infections in drug treated tumor patients has continued with the development of in vivo screens for the further exploration of the efficacy of antimicrobial agents. Work in this area is described in detail in the progress reports from Southern Research Institute.

III. Related Service Projects

The responsibilities of the Screening Section extend beyond the scheduling and evaluating of compounds submitted to CCNSC for testing in the Presumptive Screen. In order for the antitumor screen to function properly, all contract laboratories must employ tumor lines whose biological characteristics are uniform, and a uniform quality of host animals. At specified laboratories frozen tumor banks are maintained under contract, with new tumor lines dispensed both at specified intervals to each screening laboratory and when it appears that the characteristics of the tumor line being used are changing.

The need for host animals in good health and in large quantities necessitates close liaison with the Mammalian Genetics and Animal Production Section. This close liaison is reflected by certain screening contracts performing studies in the diagnosis and control of animal diseases.

IV. Proposed Course of Projects

In addition to supervising the testing of and the evaluation of the many synthetic compounds, plant and fermentation products which have been submitted to CCNSC, the Screening Section will be engaged in several diverse, but related areas of interest to the Chemotherapy Program of the NCI. Treatment route and frequency schedules will be requested and the data evaluated for those compounds which are of preclinical or clinical interest in order to ascertain their optimal dose, route of administration, frequency of treatment, and minimal toxicity to the host animal. Continued emphasis will be placed on evaluating the efficacy of compounds of interest in the Walker 256 survival system. With the projected inclusion of a slow growing solid tumor in the Presumptive Screen, protocols will be designed, preliminary testing scheduled, and data reviewed prior to recommending to the Scientific Director for Chemotherapy and the tumor system of choice. Careful contract monitoring will continue in order to expedite the achievement of these goals.

Table 1

Performance of *In Vivo* Screen

January, 1967 - December, 1967

	Preliminary Activity		Confirmed Activity*	Preliminary Activity	Confirmed Activity
	Phase I	Phase II	Phase I and Phase II	Phase III	Phase III
Synthetics	114	29	86	216	108
Plants	12	5	1	381	199
Fermentation Products**	43	20	41	73	35

Phase I = L-1210, day 2 treatment only

Phase II = L-1210, chronic treatment

Phase III = W-256 (IM tumor), chronic treatment

* = Preliminary activity confirmed in dose response testing

** = Includes animal products (NSC 700,000 series)

Table 2

Assignment of Synthetic Agents to Primary Screen

January 1, 1967 - December 31, 1967

In Vivo			In Vitro		
Amount*	Number	%	Amount*	Number	%
>2 gm.	2873	52.1	<150 mg.	209	3.8
1-2 gm.	1173	21.3			
<1 gm.	1254	22.8		—	—
Totals	5300	96.2		209	3.8

Number of compounds assigned to the Primary Screen by the Drug Evaluation Branch**	5509

* Quantity submitted to CCNSC.

** This does not indicate the total number of compounds assigned NSC numbers, since, by agreement with the Drug Development Branch, some suppliers ship their compounds directly to screeners. The latter are not reflected in this compilation.

Table 3

STATUS OF ACTIVE COMPOUNDS

During Calendar Year 1967

Status	Number of Compounds
Assigned to Step 3	201
Compounds Reviewed by the Drug Evaluation Committee	399
Assigned to Step 4A	14
Assigned to Step 4B	213
Deferred	88
Scheduled for Treatment Schedule Studies (March - December 1967)	70
Treatment Schedules Summarized (July 1, 1967 - December 31, 1967)	32

Table 4

COMPOUNDS ASSIGNED TO STEP 4A
DURING CALENDAR YEAR 1967

NSC NO.	COMPOUND NAME
71261	9*H*-Purine-6-thiol, 2-amino-9-(2-deoxy-β-*D*-*erythro*-pentofuranosyl)-, monohydrate
71795	6H-Pyrido[4,3-*b*]carbazole, 5,11-dimethyl- Ellipticine
71851	9*H*-Purine-6-thiol, 2-amino-9-(2-deoxy-α-*D*-*erythro*-pentofuranosyl)-
80087	*p*-Toluidine, α-inden-1-ylidene-*N*,*N*-dimethyl-
80439	4,7,10,13-Tetraoxahexadecane, 1,2:15,16-diepoxy-
93150	Cytosine, 1-β-*D*-arabinofuranosyl-, 2',3',5'-triacetate
104800	Galactitol, 1,6-dibromo-1,6-dideoxy-
107392	Picolinaldehyde, 5-hydroxy-, thiosemicarbazone
108836	1*H*-Pyrazolo[3,4-*d*]pyrimidin-4-ol, sodium deriv.
109229	*L*-Asparaginase
D 110372 E	
110435	Protein, unknown
D 113233	

NSC NO.	COMPOUND NAME
403169	7*H*-Pyrano[2,3-*c*]acridin-7-one, 3,12-dihydro-6-methoxy-3,3,12-trimethyl- Acronycine

Table 5

Protocol* For Initial Studies for Determination of Route and Schedule Dependency in Therapy of Leukemia L1210.

Route	Treatment Schedule
IP	Once each day from days 1-9.
IP	Once on day 1 only.
IP	Every 3 hours on day 1 only.
IP	Once each day from days 1-5.
IP	Once every 8th day; days 1 and 9.
IP	Every 3 hours every 8th day; days 1 and 9.
IP	Once every 4th day; days 1,5, and 9.
IP	Every 3 hours on every 4th day; days 1,5, and 9.
SC and oral with fasting	Once on day 1 only.
SC and oral with fasting	Once each day from days 1-9.

* All treatments are given to parallel groups of leukemic and normal mice. In addition, when requested, toxicity studies are conducted orally and IP in male and female normal mice and rats with treatments given daily for 5 days.

Table 6

List of Compounds Scheduled for Treatment Schedule Studies

1967

NSC NO.	COMPOUND NAME
744	Purine, 6-chloro-
749	*v*-Triazolo[4,5-*d*]pyrimidin-7-ol, 5-amino-
	8-Azaguanine
1026	Cyclopentanecarboxylic acid, 1-amino-
1895	Guanazole
	1,2,4-Triazolidine, 3,5-diimino-
9698	Mannitol, 1,6-bis[(2-chloroethyl)amino]-1,6-dideoxy-, dihydrochloride, *D*-
9706	*s*-Triazine, 2,4,6-tris(1-aziridinyl)-
11905	1,4-Naphthoquinone, 2-hydroxy-3-(3-methyl-2-butenyl)-
	Lapachol
13002	Acridine, 9-[*p*-(dimethylamino)anilino]-
14575	9*H*-Purine-6-thiol, 9-ethyl-
17256 E	Pregn-4-ene-3,11,20-trione, 6α-methyl-
19893	Uracil, 5-fluoro-
	Fluorouracil (USAN)
24559	Mithramycin (USAN)

NSC NO.	COMPOUND NAME
26980	Mitomycin C Carbamic acid, ester with 6-amino-1, 1a,2,8,8a,8b-hexahydro-8-(hydroxymethyl)-8a-methoxy-5-methylazirino-[2',3':3,4]pyrrolo[1,2-*a*]-indole-4,7-dione
29422	9*H*-Purine-6-thiol, 2-amino-9-β-*D*-ribofuranosyl-
29630	Glutamic acid, *N*-[3,5-dichloro-4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
31083	Actinobolin
32946	Guanidine, 1,1'-[(methylethanediylidene)dinitrilo]di-, dihydrochloride, monohydrate
38270	Antibiotic A-649-22
39147	Glutarimide, 3-[2-hydroxy-2-(5-hydroxy-3,5-dimethyl-2-oxocyclohexyl)ethyl]- Streptovitacin A
45388	Imidazole-4(or 5)-carboxamide, 5(or 4)-(3,3-dimethyl-1-triazeno)-
51095	Ammonium, trimethylpurin-6-yl- chloride
56054	Pseudourea, 2,2'-(9,10-anthrylenedimethylene)bis-[2-thio-, dihydrochloride

NSC NO.	COMPOUND NAME
56410	Carbamic acid, ester with 6-amino-1, 1a, 2,8,8a,8b-hexahydro-8-(hydroxymethyl)-8a-methoxy-1,5-dimethylazirino-[2',3':3,4]pyrrolo[1,2-*a*]-indole-4,7-dione
	Porfiromycin (USAN)
56654	Azotomycin (USAN)
	Duazomycin B
58404	Glycine, *N*-(diazoacetyl)-, hydrazide
58514	Chromomycin A3
	(Toyomycin)
61487	Carbonic acid, bis(*p*-aminophenyl) ester, dihydrochloride
65346	Sangivamycin
	7*H*-Pyrrolo[2,3-*d*] pyrimidine-5-carboxamide, 4-amino-7-β-*D*-ribofuranosyl-
68075	Thalicarpine
69856	Neocarzinostatin, crystalline antibiotic
69945	Phosphorodiamidic acid, *N*,*N*-bis(2-chloroethyl)-, compd. with cyclohexylamine (1:1)
70845	Nogalamycin (USAN)
	Crystalline Antibiotic 205t3
71261	9*H*-Purine-6-thiol, 2-amino-9-(2-deoxy-β-*D*-*erythro*-pentofuranosyl)-, monohydrate

NSC NO.	COMPOUND NAME
71795	6H-Pyrido[4,3-*b*]carbazole, 5,11-dimethyl-
	Ellipticine
71851	9*H*-Purine-6-thiol, 2-amino-9-(2-deoxy-α-*D*-*erythro*-pentofuranosyl)-
75520	Uridine, 2'-deoxy-5-(trifluoromethyl)-
77471	Mitochromin
	Mitocromin (USAN)
77472	Compound from *Saponaria officinalis*
79037	Urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-
79423	Ethanol, 2,2'-[(*p*-nitrosophenyl)imino]di-, dimethanesulfonate
82151	Daunomycin
82196	Imidazole-4(or 5)-carboxamide, 5(or 4)-[3,3-bis(2-chloroethyl)-1-triazeno]-
83265	*L*-Alanine, 3-(tritylthio)-
84423	Phenanthridinium, 3,8-diamino-5-ethyl-6-phenyl-- chloride
	Ethidium chloride
85998	Streptozotocin (Pure sample, Lot 6742)
87206	6*H*-Pyrido[4,3-*b*]carbazole, 5-methyl-
	Ellipticine, 11-demethyl-

NSC NO.	COMPOUND NAME
87974	Urea, 3-cyclohexyl-1-(2-fluoroethyl)-1-nitroso-
88104	Urea, 3-(*trans*-2-chlorocyclohexyl)-1-(2-chloroethyl)-1-nitroso-
88106	Urea, 1-(2-chloroethyl)-3-(2-norbornyl)-1-nitroso-
92188	Isoquinaldaldehyde, thiosemicarbazone
93150	Cytosine, 1-β-*D*-arabinofuranosyl-, 2',3',5'-triacetate
94100	Mannitol, 1,6-dibromo-1,6-dideoxy-, *D*-
96016	2-Naphthoic acid, 4,4'-methylenebis[3-hydroxy-, compd. with [(methylethanediylidene)-dinitrilo]diguanidine (1:1), dihydrate
100880	Camptothecin, sodium salt
	Indolizino[1,2-*b*]quinoline-7-glycolic acid, α-ethyl-9,11-dihydro-8-(hydroxymethyl)-9-oxo-, sodium salt
102816	s-Triazin-2(1H)-one, 4-amino-1-β-D-ribofuranosyl-
	5-Azacytidine
104800	Galactitol, 1,6-dibromo-1,6-dideoxy-
D104947	
107392	Picolinaldehyde, 5-hydroxy-, thiosemicarbazone

NSC NO.	COMPOUND NAME
D 113233	
403169	Acronycine 7H-Pyrano[2,3-c]acridin-7-one,3,12-dihydro-6-methoxy-3,3,12-trimethyl-
406021	9H-Purine-6-thiol, 9-β-D-arabinofuranosyl-
409962	Urea, 1,3-bis(2-chloroethyl)-1-nitroso-

Serial No. NCI-3578
1. Chemotherapy, CCNSC
2. Drug Evaluation Branch
3. Screening Section
4. Wiscon Bldg.-Kenwood
   Laboratories, Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Quantitative Evaluation of Selected Agents in the Therapy of Mouse Plasma Cell Tumors, LPC-1

Principal Investigator: D. Abraham

Co-Principal Investigators: J. M. Venditti (NIH), P. Carbone (NIH), A. Goldin (NIH), and I. Kline (Microbiological Associates, Inc., Bethesda, Maryland, under contract number PH43-64-911, Drug Evaluation Division).

Cooperating Units: Solid Tumor Service, Medicine Branch, Chemotherapy, NCI (Dr. P. Carbone); Sloan-Kettering Institute (Dr. M.Teller).

Man Years: Total - 1/2
Professional - 1/2

Project Description:

Objectives, General

Typical of plasma cell tumors, the growth of the murine plasma cell tumor LPC-1 is associated with the appearance of large quantities of abnormal protein in body fluids. Human myeloma is also characterized by the appearance of abnormal proteins. The purpose of the project is to screen new drugs in mice with LPC-1. Emphasis is placed on therapeutic response as measured by increase in lifetime, by disappearance of the abnormal protein, and the relationship between these parameters of response.

Objectives, Specific

Treatment of the rodent plasma cell tumor, LPC-1, is delayed until the tumor growth is relatively advanced and the abnormal protein can be detected in serum by electrophoresis. After a 28 day treatment period, the survival time of treated mice is compared to control mice and the protein picture of the sera for those mice surviving 40 days or more is observed. This system provides two parameters for evaluating the effects of treatment: (1) prolongation of the survival time, (2) abrogation of abnormal protein.

Methods Employed

Standard biological and biochemical laboratory procedures and new procedures developed in this program were employed.

Major Findings

Drugs which prolong the median survival time of animals, but do not markedly affect the abnormal protein have been noted, as well as drugs which affect both the median survival time and the abnormal protein concentration. The latter type drugs are investigated more fully.

A total of 18 experiments involving 28 compounds were conducted. Compounds which have produced extensive enhancement in the survival time are: NSC-94100 (Dibromomannitol), NSC-57844 (Hydracrylamide methanesulfonate), NSC-24820 (Benzaldehyde dimethanesulfonate), NSC-59640 (Benzoic acid dimethanesulfonate) and NSC-54012 (Benzaldehyde purinyl dimethanesulfonate). As indicated above, a number of dimethanesulfonates are active in this system. However, the well known dimethanesulfonate, Myleran, does not display any significant activity in this system. Further studies of the influence of treatment schedules on activity have been conducted with NSC-18429 (aniline mustard), NSC-8806 (Phenylalanine mustard) and NSC-403169 (Acronycine). These studies indicate that NSC-18429 is most effective when given as a single oral treatment. Survivors in this schedule are immune to a re-challenge by the tumor. NSC-8806 is most effective when given in a series of intermittently spaced (5 days) treatments. NSC-403169 is not effective orally and short intensive treatment seems optimal (every 3 hours on day 1 only or every 3 hours for 24 hours repeated every 4th day).

In collaboration with Dr. Morris Teller of Sloan Kettering Institute for Cancer Research a study is in progress to determine the ability of a drug which inhibits protein production in the LPC-1 system (γ_{2a} protein producer) to inhibit production of other protein types for example, γ_{2b} Bence-Jones, IgM).

Proposed Course

New agents of interest will continue to be screened in the LPC-1 assay system. Influence of treatment schedule and route on drug activity will be emphasized. Attempts will be made to determine the doubling time of the LPC-1 plasma cell. Studies of the abnormal protein-tumor-host relationship will be given considerable attention and be directed to providing information on (1) the etiology of the abnormal protein (host response or produced by the plasma cell?), and (2) the mechanism of action of drugs that affect abnormal protein production.

Publications

See current organization report of Drug Evaluation Branch.

SUMMARY REPORT

Chief, NCI, Baltimore Cancer Research Center
July 1, 1967 through June 30, 1968

Continued growth and establishment of a firm scientific base for pharmacologic and clinical experimental therapeutic studies has characterized the program this past year. The program took considerable pride in initiating its entry into the scientific community by presenting five papers at recent Spring meetings in Atlantic City. With new Sections in Immunology and Cell Biology, Radiation Therapy, and Neurosurgery to complement strong on-going efforts in Biochemistry and Medicine, it seems assured that the Baltimore program will strengthen and broaden the overall efforts of the NCI in chemotherapy of malignant disease.

The inter-relationship between the NCI and the Division of Direct Health Services at the Baltimore USPHS Hospital continues to be close and the collaborative effort has survived an approximately 50% increase in chemotherapy patients at the hospital and the opening of the basic pharmacology laboratories. Continued evolution in hospital nursing, pathology services, and administrative interaction can be anticipated in the program. New clinical care programs now range from a home-like minimal care unit where patients receive no planned nursing assistance, to an intensive therapy unit heavily supported by computer-compatible patient monitoring equipment. These units are being evaluated with regard to their importance and use in the management of patients with cancer and may serve as prototypes for other chemotherapy programs. Treatment programs remain primarily concerned with chemical control of cancer employing the newer agents, many of which are engendered entirely within the NCI-Chemotherapy program. Thus compounds which have been identified and delineated with regard to screen activity and which have been formulated and tested for toxicity and estimated tolerated dose in other additional parts of the NCI program, are brought to clinical study in Baltimore where clinical toxicity and therapeutic potential can be initially evaluated. Where of interest and pertinence, basic pharmacologic and clinical laboratory investigations have paralleled these developments.

Although the clinical program, then, is concerned with drug control of cancer, combination approaches with radiation therapy are under study and attempts are being made to establish the models and scientific base for potential immunotherapeutic adjuvant studies.

The Pharmacology Laboratory activities are growing rapidly and are increasingly approaching the "tooled-up" stage. We already face problems of limited space and animal facilities. The scientific strengths of the laboratory are becoming evident. Increased participation with clinical programs, strengthening scientific ties with other institutions in Baltimore, and additional responsibility in Pharmacology training can be anticipated for the future.

The activities of the entire program are becoming increasingly recognized in the Baltimore medical and scientific community. We have representation on the Ad Hoc Committee for Cancer in the Maryland Regional Medical Program and it is hoped that the unit can function in some way to represent the NCI in aiding this broad program in its goals of cancer control and treatment.

Serial No. NCI-6025
1. Baltimore Cancer Research Center
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: In Vitro Studies of Immunologic Reactivities in Clinical Disorders of Delayed Hypersensitivity With Particular Reference to Malignant Lymphoma

Principal Investigator: Jerome B. Block, M.D.

Other Investigators: Arthur A. Serpick, M.D.
Thomas Waldmann,M.D.
Warren Strober, M.D.

Cooperating Units: Metabolism Branch, NCI, Bethesda, Maryland
Surgery Department, USPHS Hospital, Baltimore, Maryland

Man Years (July 1, 1967 through June 30, 1968)
Total: 1½
Professional: ½
Other: 1

Project Description:

Objectives:

It has been shown that in vitro lymphocyte transformation reflects clinical expression of delayed hypersensitivity, which may in turn indicate extent of disease and clinical prognosis in a variety of disorders (sarcoidosis, Hodgkin's disease, ataxia telangiectasis, leprosy, etc.). In addition, there is evidence in experimental systems, along with suggestive clinical support, that there may be multiple populations of lymphocytes of differing reactivity which may be functionally impaired by circulating, plasma factors. To evaluate these considerations clinically, chylous and serous non-exudative effusion along with samples of thoracic duct chyle were obtained from patients with Hodgkin's disease and intestinal lymphangiectasis and the suspended lymphocytes were studied in vitro for immunologic reactivity by their transformation response to phytohemagglutinin and to streptococcal o antigen.

Major Findings:

Lymphocytes from chyle or effusions often reacted normally to in vitro mitogenic or autogenic stimulation while circulating lymphocyte transformation was impaired. In the patients with Hodgkin's disease evidence for a toxic plasma factor was found which could inhibit in vitro reactivity of lymphocytes from the effusions.

Significance to cancer research:

These studies afford increased understanding of the disease manifestations of malignant lymphoma and suggest additional approaches to their control.

Proposed course:

Continued investigation along these lines.

Honors and awards: None

Presentations:

Cell Biology Section, Federated Societies of Experimental Biology and Medicine, 1968.

Publications: None

SUMMARY REPORT

Clinical Branch, NCI, Baltimore Cancer Research Center
July 1, 1967 through June 30, 1968

The activities in the clinical areas have continued predominantly to explore the use of new chemotherapeutic agents employed singly at varied and controlled dosage schedules, or in combination with other agents of proved value. Among the new agents studied, some for the first time in an NCI program, are imidazole carboxamide, duanomycin, lapachol, and l-asparaginase. These few agents which range from natural products to synthetics have become associated with a variety of clinical toxic effects which point up the requirements of sound clinical and pharmacologic laboratory support for experimental therapeutic programs. For example duanomycin has been associated with cardiac failure and work in the laboratory has evolved a sensitive and reproducible animal model to study and control these effects. Lapachol produces a profound acute, clinical prolongation of prothrombin time which has been shown to be controlled with vitamin K. Studies are in progress now to evaluate its prolonged use and therapeutic potential.

Such new agents are being used, also, in combination or in comparative studies with such drugs as cytosine arabinoside, methotrexate, bis-β chlorethylnitrosurea, vincristine, cytoxan, natulan, prednisone, and 6-mercaptopurine. Studies are planned to evaluate the alterations in pharmacologic disposition and metabolism of single agents when used in combination with other known chemotherapeutic agents or with agents such as asprin, indomethacin, phenobarbital, ethyl alcohol, which may exert profound but unsuspected effects on drug binding, excretion, or metabolism.

Early attempts are also in progress to effectively utilize combinations of chemotherapy and x-irradiation for malignant disease. In particular, for early Hodgkin's disease there may be an unanticipated role for the combination if the long term morbid and toxic results of intensive radiotherapy limit this uni-modal approach to disease control. Plans are in addition, to develop computer techniques for isodose determinations to permit reproducible and comparable regimens of x-irradiation therapy which may, in addition, minimize morbidity and normal tissue injury.

Patient supportive care remains a major effort with a continuing program of platelet infusions and early steps being taken for frozen preparations of leukocytes and platelets for subsequent infusion. Septic and unexplained shock is under intensive study from the point of view of physiological derangements, therapy, and microbiology. It is hoped that useful data will accrue from these efforts to permit a more effective approach to this severe clinical problem. A neurosurgical program is in operation with gradually increasing referrals of patients with brain tumors for therapy with experimental chemotherapeutic agents. This new program should result in fundamental information and significant insight into the clinical management of primary and metastatic brain tumors - an area without significant therapeutic advance, with regard to patient survival, for over a decade.

Serial No. NCI 6023
1. Baltimore Cancer Research Center
2. Radiation Therapy Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Radiation Therapy Service

Principle Investigators: Kirkland C. Brace, M.D.

Other Investigators: Vernon R. Croft, M.D.

Cooperating Units: Medicine Service, Baltimore Cancer Research Center

Man Years (July 1, 1967 through June 30, 1968)
Total: 2
Professional: 2
Other: 0

Project Description:

Objectives:

To provide the optimal radiation therapy for the patients on the Tumor Service of this institution. The major effort of this Department is the investigation of the use of Chemotherapy and Radiotherapy in the treatment of malignant tumors. A large part of the facilities must be utilized in the palliation of disease and in the treatment of patients not on the Tumor Service.

Summary of Work:

The physical facilities for the Radiation Therapy Service were completed in March of 1967. We have now had one full year of operation with all facilities available. During that time 178 patients were seen in consultation for both the Tumor Service and the hospital in general. A total of 154 patients have been treated. The majority of these 138 were from the Tumor Service and the balance of 16 came from the other services of the hospital.

Methods Employed:

All the modes of radiation therapy available here were utilized in the treatment of patients. These include:

		No. of Treatments
Superficial X-Ray	70 to 120 kilovolts	14
Deep X-Ray Therapy	200 to 285 kilovolts	22
Super Voltage X-Ray	Cobalt Radiation	1804
Radium and Cesium	Interstitial and Intracavitary	8

Major Findings:

The principle study of the use of a combination of Chemotherapy and X-Ray therapy in the treatment of Hodgkin's Disease has just gotten underway. We have as yet seen no severe complications from the treatment. The response to other forms of therapy has been within the anticipated limits of the treatment.

Serial No. NCI-6024
1. Baltimore Cancer Research Center
2. Neurosurgery Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Development of the Neurosurgical Service With the Primary Interest in the Treatment of Brain Tumors at NCI, BCRC

Previous Serial Number: None

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Byron Hurwitz, M.D.

Cooperating Units: Medicine Service, NCI
NCI-LCP

Man Years
Total: 1½
Professional: 1½
Other: 0

Project Description:

Objectives - Major:

1. The development of the Neurosurgical Service with the primary interest in the treatment of brain tumors.
2. The development of pharmacology laboratory facilities for the broad evaluation of chemotherapeutic treatment of brain tumors.

Objectives - Minor:

1. The development of the Brain Tumor Chemotherapy Study Group, as Co-chairman, by participation in meetings, visits to various neurosurgical centers in the country, protocol development and evaluation of the data generated therefrom.
2. Continued attempts toward the development of a transplantable monkey brain tumor, in conjunction with Dr. Margaret K. Kelly, at NIH-NCI-LCP.
3. Evaluation of intrathecal chemotherapy via relatively standardized techniques in monkeys.
4. Drug disposition in elasmobranchs, and particularly, within the central nervous system.
5. Supervision and consultation with the neuropharmacology program for the development of repetitive ventricular perfusions at A.D. Little, Co., Boston, Mass.
6. The distribution and metabolism of steroids in the cat brain as a joint project with members of the National Heart Institute.

Methods Employed:

Referrals to the Center of patients with brain tumors are being cultured, so as to maintain a reasonable number of brain tumors on chemotherapy at any one time. This, however, will be slow to develop.

Initial investigations in patients with malignant brain tumor are in the use of BCNU, 50-60 mg/M^2 weekly, for six plus weeks.

Major Findings:

Listed below are the data related to patients cared for by the Section of Neurological Surgery, at BCRC, from October 1967 through March 1968. The majority of patients cared for and operated upon have been outside the Brain Tumor Chemotherapy Program, and have been seen as a service to the PHS Hospital.

MONTH 1967	NO. PAT. ATTENDED	NO. INPAT. CONSULTS	NO. OUTPAT. CONSULTS	OPERATIONS & SURG. PROC. PERFORMED (Diag. & minor proc. not incl.)	PAT. ADMITTED TO TUMOR-Neuro SERVICE
Oct.	6	4	2		
Nov.	11	4	2	8	
Dec.	4	3		2	
1968					
Jan.	10	4	3	1	4
Feb.	9	4	2	1	4
March	20	12	4	5	2
TOTALS	60	31	13	17	10

Proposed Course:

Continued accession of patients into the study and development of laboratory support and facilities.

Publications and Presentations :

1. Walker, M.D., Adamson, R.H., Oliverio, V.T., Hahn, M.A. and Rall, D.P.: The Disposition of Methotrexate, Inulin, Antipyrine and Cortisol in Elasmobranchii. The Pharmacologist 9,2: 241, Fall, 1967.

(Publications and Presentations continued)

2. Walker, M.D., Dalgard, D.W.: The Toxicity of Intrathecal Cytosine Arabinoside (Ara-C) and Phenylalanine Mustard in Monkeys. Proceedings 9: 99, March 1968. (abstract only)

3. Walker, M.D., Carleton, F.J. and Owens, E.: The Distribution of Tritium After Administration of Mithramycin-H^3 to Normal and Brain Tumor Bearing Monkeys. Fed. Proceedings 27,No.2,: 760, March,April 1968.

4. Bering, E.A.,Jr., Rall, D.P., Walker, M.D., Leventhal, C. and Ommaya,A.: Intrathecal Chemotherapy of Gliomas: Rationale and Current Status. Presented at The New York Academy of Sciences Conference on Research in the Experimental and Clinical Aspects of Brain Tumors, February, 1968.

5. University of Maryland - Cancer Therapy Lecture Series, March 3, 1968: Different Approaches to the Chemotherapy of Brain Tumors.

6. USPHS Medical Librarian School, March 20, 1968: A Survey of Neurological Surgery.

SUMMARY REPORT

Medical Service, Baltimore Cancer Research Center

The Medical Service serves as an integrated clinical and laboratory training and research center for cancer and occupies an entire floor of the United States Public Health Service Hospital in Baltimore, a general hospital with approved residencies in the major medical specialties. The inpatient service numbers 75 beds, and patients are screened and selected on a referral basis and are all available for clinical studies. The vast majority of patients are referred from physicians in private practice, and those associated with hospitals and clinics. Approximately 250 patients were admitted for study during the past year, of which the acute leukemias and Hodgkin's disease constitute the largest disease entities, numbering 70 and 35 patients respectively. Training is provided at all levels--from medical student and resident to post-doctoral fellow. The Medical Service has undertaken a responsible position in the community by providing consultation services in medical oncology for several community hospitals in Baltimore, as well as the University of Maryland, and by utilizing its clinical resources in training of medical and paramedical personnel from these institutions. This sense of awareness in the community has stimulated the participation of the Medical Service of the Baltimore Cancer Research Center in several important regional committees--the Professional Educational Committee, Baltimore City Chapter of the American Cancer Society, the Medical Advisory Steering Committee of the Baltimore Chapter, Red Cross Blood Program and to the Sub-Committee on Blood Banking of the Ad Hoc Advisory Committee on Cancer of the Regional Medical Program of Maryland.

The program emphasis is on control of cancer by chemical means, an assessment of the tumor eradication capacity of agents, design and conduct of toxicologic and pharmacologic drug studies, and study of tumor-host interaction. There is a multidisciplinary approach to therapeutic trials involving chemotherapy, radiotherapy and immunotherapy. Maximum patient support is provided by means of platelet and granulocyte replacement therapy. The Baltimore Red Cross is collaborating by providing platelets and the antihemophiliac cryoprecipitates prepared as a by-product of platelet separation are provided for the community at large. Further areas of research include cryopreservation and improved separation of blood components, as well as homologous and autologous bone marrow replacement. Specific clinical research projects include studies of microcirculation, immunologic competency, bone marrow and tumor cell kinetics and a search for biochemical parameters of disease activity in leukemia.

There is a longitudinal study of the physiologic derangements in patients with progressive leukopenia, fever, sepsis and shock in an attempt to define the natural history of septic shock and conduct therapeutic trials in a more controlled population. For these purposes, a custom-made and installed automatic physiologic monitoring system has been placed in an Intensive Care Unit, and which is said to be the most sophisticated monitoring system on the East Coast. These modern facilities improve patient care and nursing efficiency, and will enable analysis of the efficacy of various therapeutic regimens in critically-ill patients. In such patients vital signs are continuously monitored, as well as cardiac output, oxygen consumption and veno-arterial shunting. Anaerobic metabolism and perfusion efficiency are estimated from arterial and venous blood gases, and lactate and pyruvate concentrations. Reticuloendothelial function is studied by clearance of aggregated albumin. Microvascular rheology is observed by fluorescein retinography and quantitated by equilibration of labeled red and white blood cells. The mass of data generated by these studies is processed on digital computers.

A clinical and experimental renal physiology laboratory is conducting a program of investigation relating neoplastic disease and pharmacologic agents to renal function. Automated and analytical facilities and electronic data processing allow the design and performance of serial and prolonged studies of kidney function.

Basic laboratory research activities related to pharmacologic, biochemical and immunologic studies of chemotherapeutic agents.

A great deal has been accomplished during the year. The expanded clinical and laboratory resources are now "tooled up" and attacking fundamental areas in cancer treatment. The problem of adult acute leukemia appears quite complex despite impressive progress in terms of remission induction. The possible role of serum muramidase as a biochemical parameter reflecting leukemia despite "complete hematologic remission" may increase an understanding of the kinetics of this disease and how distant we are from achieving complete eradication of leukemic cells. Impressive results have been achieved in chemotherapy of Hodgkin's disease (advanced), and these principles are being applied to patients with localized disease. The approach to treatment of slow growing tumors by intermittent therapy and utilization of patient survival as a parameter of response to chemotherapy are direct clinical applications of recently acquired experimental data.

Serial No. NCI-6002
1. Baltimore Cancer Research Center
2. Medicine Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Clinical Significance of Serum and Urinary Muramidase Activity in Leukemia and Other Hematologic Malignancies

Previous Serial Number: None

Principal Investigator: Peter H. Wiernik

Other Investigators: Arthur A. Serpick

Cooperating Units: None

Man Years (July 1, 1967-June 30, 1968):

Total:	2
Professional:	2
Other:	0

Project Description:

Objectives:

To determine whether or not pre-treatment and serial serum and urine muramidase determinations might reveal clinically occult persistent disease activity during remission in acute leukemia. To determine whether or not pre-treatment serum muramidase levels might be of diagnostic and/or prognostic significance. To determine whether or not serial muramidase determinations throughout the course of acute leukemia might yield information useful in the management of the patient.

Methods Employed:

Serum, urine and occasionally cerebrospinal fluid muramidase were assayed by a turbidimetric method utilizing a suspension of lyophylized Micrococcus lysodeikticus as substrate and crystalline egg-white muramidase as standard. Serum muramidase levels were correlated with routine hematologic data including CBC and bone marrow examinations.

Major Findings:

1. Pre-treatment serum muramidase levels have diagnostic significance in acute leukemia. Acute lymphocytic, myelocytic, myelomonocytic, and

monocytic leukemia can usually be separated one from another by the pre-treatment serum muramidase level.

2. Pre-treatment serum muramidase levels are of prognostic significance. Those patients with the highest initial serum muramidase level (acute monocytic leukemia patients) had the poorest survival of all adult acute leukemia patients.

3. Serial serum muramidase determinations are useful in following the course of patients with acute lymphocytic, myelomonocytic, or monocytic leukemia. In these diseases, serum muramidase almost always normalizes at the time of complete remission. Rarely serum muramidase may become normal for as long as 1 week before bone marrow evidence of complete remission is obtained. In acute myelocytic leukemia, the results were variable and, therefore, serial determinations in this disorder are of no help.

Significance to Cancer Research:

Measurement of serum and urinary muramidase in acute lymphocytic, myelomonocytic, and monocytic leukemia provides a biochemical parameter by which disease activity may be measured in these disorders. By use of this parameter, it may be possible to more accurately diagnose remission and relapse in these diseases. Hence, the results of chemotherapy may be better evaluated.

Proposed Course:

Continue early studies.

Honors and Awards: None

Publications:

1. Wiernik, P.H. and Serpick, A.A.: Serum and Urine Muramidase Activity in Acute Leukemia. Proceedings of the American Society for Clinical Oncology, April 10, 1968.

2. Wiernik, P.H. and Serpick, A.A.: Clinical Significance of Serum and Urinary Muramidase Activity in Leukemia and Other Hematologic Malignancies (in press) Amer. J. Med.

Serial No. NCI-6003
1. Baltimore Cancer Research Center
2. Medicine Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Mechanism of Glucocorticoid-Induced Regression of Lymphoid Tissue

Principal Investigator: Peter H. Wiernik

Other Investigators: None

Cooperating Units: None

Man Years (July 1, 1967-June 30, 1968):

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

Objectives:

The principal investigator has shown that increased acid ribonuclease activity is found in normal and neoplastic lymphoid tissue prior to regression induced by glucocorticoids. It is not clear whether this increased ribonuclease activity is a primary or secondary function of corticosteroid effect on lymphoid tissue. Others have shown that an early effect of glucocorticoid administration to intact animals is increased hepatic tyrosine-alpha ketoglutarate transaminase activity, and have suggested that the product of this reaction, glutamate, may be the mediator of glucocorticoid influence on lymphoid tissue. The injection of intraperitoneal glutamate to adrenalectomized animals has been reported to cause involution of normal lymphoid tissue. The purpose of this study is to determine whether or not ribonuclease induction in lymphoid tissue is necessary for glucocorticoid mediated regression. The involution of lymphosarcoma P1798 under the influence of glucocorticoid has been shown to be prevented by the simultaneous administration of Puromycin. No induction of ribonuclease activity occurs in P1798 lymphosarcoma when glucocorticoid and puromycin are given concurrently. Experiments will now be done to determine whether or not glutamate causes regression of P1798 lymphosarcoma. If such a regression occurs, the effect of glutamate plus puromycin will be investigated in this mouse tumor system.

Major Findings:

Studies just initiated.

Significance to Cancer Research:

These studies are designed to shed some light on the mechanism by which glucocorticoids cause lympholysis. Such information may be important in understanding the influence of an organism hormonal internal environment on normal and neoplastic lymphoid tissue growth.

Proposed Course:

Continue early studies.

Honors and Awards: None

Publications: None

Serial No. NCI-6004
1. Baltimore Cancer Research Center
2. Medicine Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Platelet Transfusion Therapy

Principal Investigators: Arthur A. Serpick and Walter R. Miller

Other Investigators: Paul K. Johnson and Lewis M. Slater

Cooperating Units: Blood Bank, USPHS Hospital; Baltimore Red Cross; Leukemia Task Force, NCI

Man Years

Total:	4 1/3
Professional:	1 1/3
Other:	3

Project Description:

Objectives:

It has been demonstrated that in patients with acute leukemia platelet replacement therapy can be repeatedly effective in increasing the circulating platelets and arresting hemorrhage. Investigations to determine the most efficient methods of platelet separation, modification and administration were performed. The major limitation of platelet therapy is their short storage time. The development and assessment of adequate methods for preserving platelets is a prime objective of this project.

Major Findings:

A comparative study of two centrifuge models revealed similar platelet yields/unit. Acidification of the platelet rich plasma was necessary to prevent irreversible clumping of the platelet concentrates. A randomized paired study of the use of a commercially available filter during the administration of platelet concentrates did not show any deleterious effects on the post-transfusion platelet increment. Patients with multiple previous platelet transfusions, splenomegaly or fever appear to have lower post-transfusion increments. Recipient reactions have been less than 10% and consist predominantly of chills, fever, and occasional urticaria.

Significance to Cancer Research:

The constant threat of bleeding in the patient with leukemia has been reduced. The physician has a greater therapeutic index in the administration of myelosuppressive drugs with effective platelet replacement therapy. Cryopreservation of platelets may be a feasible approach to solving the logistical problems of platelet storage and availability.

Honors and Awards: None

Publications:

1. Miller, Walter R. and Serpick, Arthur A.: Platelet Separation and Transfusion on a Cancer Chemotherapy Service. Submitted for Presentation, XII Congress, International Society of Hematology.

2. Serpick, Arthur A. and Miller, Walter R.: The Use of Platelet Transfusion in the Treatment of Leukemia, in press, Maryland State Medical Journal.

Serial No. NCI-6005
1. Baltimore Cancer Research Center
2. Medicine Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Effect of Alkylating Agents on the Renal Concentrating Mechanism of Man

Principal Investigator: T. H. Steele

Other Investigators: J. B. Block and A. A. Serpick

Cooperating Units: None

Man Years

Total:	1/3
Professional:	1/6
Other:	1/6

Project Description:

Objectives:

To determine whether alkylating agents administered to man can cause functional changes analagous to those observed in viable membrane preparations *in vitro*.

Methods Employed:

Parameters of renal function were studied before and after the acute administration of alkylating agents to patients according to NCI chemotherapy protocols.

Major Findings:

Patients, in water diuresis, who received a single large dose of Cyclophosphamide (1500 mgs./M^2 BSA) showed an abrupt decrease in urine volume and free water clearance, without concomitant change in glomerular filtration rate or rate of solute excretion. In addition, parameters of electrolyte excretion remained unchanged. This effect was of rapid onset and continued in essentially stable fashion for at least two hours after the administration of cyclophosphamide. In contrast, subjects who received Cyclophosphamide during antidiuresis (achieved through the infusion of pitressin) fail to show any modification of the above parameters of renal function.

Significance to Cancer Research:

Nitrogen mustard and non-nitrogen mustard appeared to have an effect antagonistic to antidiuretic hormone in the viable toad bladder preparation *in vitro*. In contrast, the studies reported above appear to show an opposite result--that is, an action of an alkylating agent precursor which either mimics that of antidiuretic hormone, or stimulates the release of endogenous hormone.

Proposed Course:

Continued studies using other alkylating agents.

Honors and Awards: None

Publications:

J. B. Block, A. A. Serpick and T. H. Steele: An Acute Effect of Cyclo-Phosphamide on the Renal Concentrating Mechanism of Man. *Clinical Research* (in publication).

Serial No. NCI-6006
1. Baltimore Cancer Research Center
2. Medicine Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Alterations in Mineral Metabolism During the Renal Response to Volume Expansion in Man

Principal Investigator: T. H. Steele

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1/3
Professional:	1/6
Other:	1/6

Project Description:

Objectives:

Saliuretic and calciuric responses to expansion of the extracellular space have been noted previously. In contrast, however, the regulation of phosphate excretion has been believed to lie solely within the domain of parathormone action.

Methods Employed:

The excretion of phosphate was studied in 12 subjects undergoing rapid volume expansion with isotonic saline.

Major Findings:

While inulin clearance and filtered load of phosphate did not change significantly during rapid expansion of the extracellular space in these subjects, phosphate excretion and fractional excretion nearly doubled. During this interval, there was a threefold increase in the rate of sodium excretion.

Significance to Cancer Research:

Hypercalcemia is a complication of many forms of neoplastic disease. Although expansion of the extracellular space *per se* has been shown to

Significance to Cancer Research (cont'd):

result in an increased rate of urinary calcium excretion, the present data suggest that a significant augmentation of phosphate excretion is also a part of the response to volume expansion. Hence, a physiologic mechanism appears to exist whereby calcium and phosphate excretion are simultaneously promoted, in the absence of hypercalcemia. The data suggest that factors other than parathyroid activity may be of importance in the regulation of renal phosphate excretion.

Proposed Course:

It is planned to extend the above studies by incorporating results from patients with hypercalcemia of diverse etiologies. In addition, parameters of calcium and magnesium metabolism will be concurrently investigated, using atomic absorption spectroscopy.

Honors and Awards: None

Publications:

1. T.H. Steele: The Phosphaturic Effect of Volume Expansion. Clinical Research (in publication).

Serial No. NCI-6007
1. Baltimore Cancer Research Center
2. Medicine Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: A Study of the Seriously-Ill Patient with Cancer

Previous Serial Number: None

Principal Investigator: W. Leigh Thompson

Other Investigators: Gerald Vermeulen, Barry Neidorf and Robert Wainer

Cooperating Units: None

Man Years

Total:	3
Professional:	2
Other:	1

Project Description:

A multidisciplinary approach to the seriously-ill, usually septic, cancer patient is being established to provide prognostic and therapeutic indices for improved care. Hemodynamic, respiratory, reticuloendothelial, renal, hematologic, and metabolic changes in patients progressing from fever to sepsis and shock will provide a basis for delineating the pathophysiology of septic shock in these and other patients. New drugs being tested include hydroxyethyl starch, dopamine, sodium acetate solutions, and propranalol. Sophisticated automated monitoring equipment displays and records continuously arterial pressure, venous pressure, heart rate, temperature and respiratory rate of one to eight patients. Automated data processing is being used to obtain, collate, store and analyze data from laboratory and clinical sources.

Objectives:

1. To install and utilize automated monitoring equipment.

2. To train available nursing personnel in the use of monitoring equipment and in skilled management of the seriously-ill patient.

3. To write efficient data collation and statistical analysis computer programs.

Objectives (cont'd):

4. To establish methods of measuring hemodynamic, respiratory, reticuloendothelial, renal, hematologic, and metabolic parameters.

5. To study the effects of routine potassium supplementation on strength, arrhythmias, and renal function before and after development of fever and sepsis.

6. To study the effects of routine digitalis administration in febrile patients on hemodynamic and metabolic parameters during sepsis and shock.

7. To evaluate the efficacy of hydroxyethyl starch, a new plasma substitute, in blood volume expansion and to compare its microvascular hemorheological properties with those of medium and low molecular weight dextran.

8. To compare the hemodynamic, renal and other effects of dopamine, isoproterenol, mephentermine and metaraminol in patients with shock.

Methods Employed:

An Astrup apparatus and motorized Natelson microgasometer are used routinely to measure arterial and central venous blood pH, PCO_2, PO_2 and oxygen content. Expired air is collected in Douglas bags and analyzed as it is transferred to a Tisot spirometer. The multiple corrections and calculations necessary to correct blood gas data for patient temperature, etc. are calculated from multiple regression analysis of published data and the computer generates derived parameters as needed. Creatinine and urea clearances, sodium, potassium and osmolar clearances are routinely measured daily on patients with sepsis admitted to this study. Special coagulation tests are made when disseminated intravascular coagulation seems likely and skin testing with epinephrine is used to detect "endotoxemia". Microaggregated albumin tagged with I-131 will be used to measure RES activity.

Major Findings:

In the development of shock and hypotension in patients with proven or presumed sepsis, hypokalemia and hypocalcemia are prominent electrolyte abnormalities and may figure significantly in potentiating the neuromuscular blockade of antibiotics. The "hyperdynamic" type of septic shock seems to predominate in this patient population and responds briefly to isoproterenol, but several patients have now survived a prolonged period of profound septic shock. From our first experience, dopamine seems to be a reliable new agent with less arrhythmogenic potential than isoproterenol and hydroxyethyl starch continues to provide predictable volume expansion without side reactions. Sophisticated automated monitoring equipment has been installed and is in operation, although considerably more time is required in nursing education to optimally utilize this type of instrumentation.

Major Findings (cont'd):

Multiple general-purpose computer programs have been written to make possible data collection, collation and analysis.

Significance to Cancer Research:

Most patients die of infections and most of these progress orderly from fever to sepsis to shock. If we can define the sequence of events during this progression, we may be able to institute more specific and effective therapy earlier before generalized ischemia compromises recovery. Automated monitoring equipment will help, in part, compensate for the shortage of competent nursing personnel in the care of the seriously-ill patient.

Proposed Course:

We will begin the rapid data-collection phase of this multiparametric study at this time now that the methods have been developed. Specific animal testing of several aspects of the septic shock study may be performed. Studies of special interest include the effects of antibiotics on calcium and potassium dynamics and the effects of metabolic and respiratory acidosis and alkalosis on responsiveness to dopamine, isoproterenol, norepinephrine and other sympathomimetics.

Honors and Awards: None

Publications: None

Serial No. NCI-6008
1. Baltimore Cancer Research Center
2. Medicine Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Effect of Changes in the Extracellular Space on Renal Uric Acid Reabsorption in Man

Principal Investigator: T. H. Steele

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1/3
Professional:	1/6
Other:	1/6

Project Description:

Objectives:

To further define the role of extracellular volume changes in regulating reabsorption of solutes other than sodium by the kidney.

Major Findings:

A group of eight patients and five normal volunteers were studied, using clearance techniques during vigorous volume expansion with isotonic saline. While inulin clearance and calculated filtered load of urate did not rise significantly, the plasma urate concentration and excretory rate decreased in like proportions. Therefore, the ratio of urate to inulin clearance (C_{urate}/C_{inulin}) remained unchanged.

Five of the above subjects and five additional individuals were treated 90 minutes prior to initiating study with pyrazinamide, a potent anti-secretory agent, which predictably depresses urinary urate excretion approximately 80% through inhibition of renal tubular secretion, In contrast to the foregoing, vigorous expansion of this group of subjects resulted in a marked increase in urate excretion and a significant increase in the urate to inulin clearance ratio. This increase in urate excretion, induced by volume expansion, was accompanied by large increases in sodium excretion.

In a third group of ten subjects pretreated with pyrazinamide, ethacrynic

Major Findings (cont'd):

acid was administered in order to cause acute extracellular volume depletion. In five of these subjects, the losses of sodium and water were replaced by the concurrent administration of intravenous saline. In the subjects who became volume depleted, the urate excretory rate and the urate to inulin clearance ratio decreased markedly, whereas saline repletion of the extracellular space prevented this effect.

These data indicate that uric acid reabsorption varies with changes in volume of the extracellular space, in a manner similar to sodium. This effect is not normally noted because, in the normal individual, 80% of the urinary urate excreted is derived from tubular secretion. However, during pyrazinamide inhibition of secretion, a consistent effect of volume depletion and expansion on fractional excretion of filtered urate is noted. Although the magnitude of this effect is variable, it appears to be quite large in some individuals.

Significance to Cancer Research:

Further knowledge of the processes concerned in the regulation of renal uric acid transport is desirable in order to allow a more adequate evaluation of and rational approach to therapy of uric acid nephropathy.

Proposed Course:

Project is completed. Manuscript to be submitted for publication.

Honors and Awards: None

Publications:

1. T. H. Steele: The Latent Effect of Volume Expansion on Urate Reabsorption in Man. Clinical Research (in publication).

Serial No. NCI-6009
1. Baltimore Cancer Research Center
2. Medicine Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Clinical Trials of Chemotherapeutic Agents in Patients with Cancer

Principal Investigator: Arthur A. Serpick

Other Investigators: Jerome B. Block, Lewis Slater, Peter Wiernik, Paul Johnson, Allen Bredt, Kirkland Brace, Michael D. Walker, Edward Henderson, Vincent DeVita

Cooperating Units: Neurosurgical, Radiotherapy and Immunology Sections, Medicine Branch, NCI; Acute Leukemia B Group

Man Years (July 1, 1967-June 30, 1968):

Total:	12
Professional:	10
Other:	2

Project Description:

Objectives:

The principle objective of this project is to improve the chemical control of human cancer. The emphasis is on the evaluation of new agents, as well as improvement in the use of conventional drugs, singly or in combination, the design and conduct of toxicologic and pharmacologic drug studies and the study of tumor-host-drug interaction. There is a multidisciplinary approach to therapeutic trials involving chemotherapy, radiotherapy and immunotherapy.

Major Findings:

Acute Leukemia

Adults

The antibiotic daunomycin has demonstrated significant antileukemic activity and seems to be the most potent active single agent in acute myelogenous leukemia. In collaboration with the Acute Leukemia B Group, Protocols #6706 and #6706/A were designed to determine the relative remission rates and relative toxicities of various schedules of administration of this agent. Among 45 patients evaluated at this institution alone a 3-5 day course of

Major Findings Cont'd:

this drug appears optimum with remission rates of 55% compared to 20% and 15% respectively for bi-weekly and weekly drug administration. This agent is very myelosuppressive, and extreme and prolonged granulocytopenia all too frequently result in a fatal sepsis. Patients achieving remission are randomized into two different combination maintenance regimens--6-mercaptopurine + methotrexate versus cytosine arabinoside + methylglyoxal-bis-guanylhydrazone (Methyl-gag)--all agents possessing some activity in acute myelogenous leukemia. Thus far remissions are equally short in both regimens, indicating a major obstacle towards treatment of this disease.

A recently initiated protocol study entails a comparison of the most optimum schedule of daunomycin with the previously successful Intensive Combination Drug Therapy--POMP (vincristine, prednisone, methotrexate and 6-mercaptopurine) as to frequency of remission, length of remission, and morbidity of drug therapy. Maintenance regimens involve randomization to an unmaintained group, a non-cycle dependent drug (Cyclophosphamide) and a cycle-dependent drug that has been demonstrated to have antileukemic activity in an intermittent schedule (Hydroxyurea).

Following the initial favorable results with cytosine arabinoside, given by daily injections, this drug is now being administered by short-term infusion courses in collaboration with the Leukemia Service at the Medicine Branch of the National Cancer Institute in Bethesda. The drug is administered so as to insure significant plasma levels for four hours out of each 24 hours during each course. Patients are randomly assigned to a.) a single four-hour infusion daily for four days or b.) two infusions of two hours' duration at 12-hour intervals for four days. Thus far, remission rates approximate 40% with no discernible difference between the two schedules employed. The morbidity and mortality for these drug regimens appear to be significantly less than that of other drugs with a fatality rate as low as 15%.

A combination of daunomycin, vincristine and prednisone exhibited a striking activity in adults with acute lymphocytic leukemia, and 5 consecutive adults with this form of disease easily achieved remission. Such patients are now being randomized between this combination and POMP.

Children

The remission-induction potential of daily versus intermittent four-day courses of prednisone used in conjunction with vincristine was assessed and appears to be comparable in both antileukemic activity and corticosteroid side effect. Subsequent consolidation and maintenance therapy for these children involved a comparison of regimens combining a potent S phase inhibitor (methotrexate) with a non-S phase inhibitory alkylating agent (Cyclophosphamide). While this study is still in progress, it appears that the duration of remissions is shorter than that of the previous POMP program.

Major Findings Cont'd:

Daunomycin has been characterized as a remission-induction agent in children with leukemia and, in combination with vincristine and prednisone, rapidly produces substantial remissions.

Lymphoma

Continued follow-up of patients with advanced Hodgkin's Disease treated with a combination of four active agents--Natulan, Nitrogen Mustard, Vincristine and Prednisone continue to show a long duration of unmaintained remission with successful reinduction on relapse with these same agents. Upon completion of six courses of therapy patients are now randomized to a maintained versus an unmaintained group.

Success with this combination in patients with advanced Hodgkin's Disease has prompted a current study in localized nodal disease, where chemotherapy is compared with tumoricidal radiotherapy in its ability to prevent recurrent disease in adjacent, clinically uninvolved lymph nodes. Thus far, it has been shown that the sequence of radiotherapy followed by intensive chemotherapy, as well as the reverse sequence, produces no undue morbidity.

Cyclophosphamide therapy employed as large intravenous dosages every third week has had considerable success in treatment of patients with Burkitt's tumor and such intermittent schedules in transplantable animal tumors indicate that such regimens possess greater therapeutic activity and a wider margin of safety than with daily treatment at maximum tolerated dosages. Such schedules have been applied clinically in patients with lymphosarcoma, and reticulum cell sarcoma and doses of 1500 mg/M^2 of body surface can be given repetitively every three weeks without cumulative toxicity for over a year. While the remission rate appears to be comparable to more conventional methods of administration of this agent, the duration and quality of remission appears longer at first glance.

Solid Tumors

A combination of the antibiotics mithramycin and actinomycin D in patients with embryonal cell testicular neoplasms was shown to be tolerable, but offers little in the degree of remissions induced than when these same agents are used individually. Large doses of methotrexate (5 mg/kg/day) for those patients with choriocarcinoma elements in these tumors will only infrequently, and then only briefly induce tumor regression as monitored by serial urinary chorionic gonadotrophin determinations.

Cyclophosphamide in the schedule noted previously is being employed in patients with solid tumors of varying responsiveness and generation times (lung, breast, ovary, melanoma) to assess the therapeutic benefit and advantages such a regimen might have over conventional drug administration. Activity thus far has been noted in breast carcinoma and melanoma.

New Agents

Lapachol, a naphthoquinone with activity in the Walker 256 screen but inactive in Leukemia L1210, is undergoing a Phase I Study. In structure, as well as in clinical trials, this drug was found to be a Vitamin K antagonist producing depression of the Vitamin K dependent clotting factors at doses greater than 3 grams per day. This hypoprothrombinemia is rapidly reversed by the administration of Vitamin K, but the antitumor activity is unchanged in the Walker model with Vitamin K administration. Current therapeutic trials in man employing the combination administration of Vitamin K and Lapachol in long-term studies to redefine the toxicity and neoplastic activity are in progress.

Imidazole Carboxamide dimethyl triazeno in a Phase I Study was found to have a maximally tolerated dosage of 200 mg/M^2 when given in repetitive five-day I.V. courses and greater than 400 mg/M^2 when given at twice a week schedules. Regression has been noted in 3 of 10 patients with disseminated melanoma, thus confirming the similar activity noted by other groups.

Daunomycin in solid tumor trials was found to be tolerable at a weekly dose of 80 mg/M^2, and in one woman with a metastatic choriocarcinoma refractory to other agents was observed to have striking, although temporary, disease regression.

The enzyme L-Asparaginase has recently been introduced into clinical trial in patients with acute leukemia. Treatment of malignant neoplasms with this agent may represent a clear example of therapy based on a specific nutritional difference between certain malignant and normal cells. Prior to therapy the patient's leukemic cells are subjected to predictive *in vitro* tests for L-Asparaginase sensitivity. Thus far, two patients with acute myelogenous leukemia and one patient with blast crisis of chronic myelogenous leukemia have not responded to this agent.

Publications:

1. Serpick, A. A. and Henderson, E. S.: Observations on Toxicity and Clinical Trials with Daunomycin, *Path.-Biol.*, *15*:909-912, 1967.

2. Burke, P. J., Serpick, A. A., Carbone, P. P. and Tarr, N.: A Clinical Evaluation of Dose and Schedule of Administration of Cytosine Arabinoside (NSC-63878), *Cancer Res.*, *28*:274-279, 1968.

3. Henderson, E., Serpick, A., Leventhal, B. and Henry, P.: Cytosine Arabinoside Infusions in Adult and Childhood Acute Myelocytic Leukemia, *Proc.Amer.Assoc. Cancer Res.*, *9*:29, 1968.

4. Lusch, C. J., Serpick, A. A. and Slater, L.: Antipyretic Effect of Indomethacin in Patients with Malignancy, *Cancer*, in press.

LECTURES AND PRESENTATIONS

Medical Service, NCI, Baltimore Cancer Research Center

1 May 1967-30 April 1968

Dr. Serpick

September 14, 1967

Principles of Cancer Chemotherapy - Hospital Staff Conference, South Baltimore General Hospital, Baltimore, Md.

September 22, 1967

Cancer Chemotherapy and the Internist - Medical Staff Conference, St. Agnes Hospital, Baltimore, Md.

May 16, 1967

Chemotherapy of Head and Neck Tumors - Symposium of Maryland Ear, Nose and Throat Society, Baltimore, Md.

June 20, 1967

Chemotherapy of Cancer - Medical Record Librarian School, USPHS Hospital, Baltimore, Md.

July 10, 12, 17, 24

Orientation Lectures in Chemotherapy - Medical and Nursing Staff, USPHS Hospital, Baltimore, Md.

October 9, 1967

Chemotherapy of Lymphoma - Hematology Conference, University Hospital, Baltimore, Md.

December 6, 1967

Principles of Cancer Chemotherapy - Hospital Staff Conference, Kimborough Army Hospital, Fort Meade, Md.

Lectures and Presentations Cont'd:

December 11, 1967

Summary of Clinical Trials with Daunomycin - at Clinical Trials Task Force Meeting, Bethesda, Md. (NIH)

January 17, 1968

Cancer Chemotherapy - Post-Graduate Course - Advances in Medical Science, University of Maryland, Baltimore, Md.

Feburary 12, 1968

Summary of Clinical Trials with Lapachol and Imidazole Carboxamide at Clinical Trials Task Force Meeting, NIH, Bethesda, Md.

February 27, 1968

Use of Platelet Transfusion in Management of the Patient with Acute Leukemia - Seminar on Blood Component Therapy, Sponsored by Maryland Medical and Chirurgical Faculty - Johns Hopkins Hospital, Baltimore, Md.

March 15, 1968

Chemotherapy of Melanoma - Conference on Chemotherapeutic Agents in Dermatology, NIH, Bethesda, Md.

April 10, 1968

Serum and Urine Muramidase Activity in Acute Leukemia (co-author) - American Society of Clinical Oncology, Atlantic City, N.J.

April 12, 1968

Cytosine Arabinoside Infusions in Adult and Childhood Acute Myelocytic Leukemia (co-author) - American Association for Cancer Research, Atlantic City, N.J.

April 19, 1968

Chemotherapy of Solid Tumors - Hospital Staff Conference, Union Memorial Hospital, Baltimore, Md.

Lectures and Presentations Cont'd:

Dr. Thompson

February 28 and March 13, 1968

Physiology and Management of Shock - Nursing Staff, USPHS Hospital, Baltimore, Md.

March 20, 1968

EKG Interpretation - Nursing Staff, USPHS Hospital, Baltimore, Md.

March 27 and April 3 and April 10, 1968

Cardiac and Respiratory Arrest; Physiologic Monitoring - Nursing Staff, USPHS Hospital, Baltimore, Md.

April 24 and 25, 1968

Inhalation Therapy - Nursing Staff, USPHS Hospital, Baltimore, Md.

Dr. Bluming

December 14, 1967

Burkitt's Tumor - Medical Staff, St. Joseph's Hospital, Towson, Md.

Dr. Wiernik

April 10, 1968 - Serum and Urine Muramidase Activity in Acute Leukemia. American Society of Clinical Oncology, Atlantic City, N.J.

SUMMARY REPORT

Laboratory of Pharmacology, NCI, Baltimore Cancer Research Center
July 1, 1967 through June 30, 1968

The new Pharmacology Laboratories are rapidly nearing functional and physical completion with activities at almost peak levels in Biochemistry and Immunology. With planned installation of an electron microscopy laboratory the major physical alterations will have been completed. The considerable problems of initiating research activities of outstanding quality with respect to instrumentation, animal facilities, supporting services, administrative practices, and required personnel appear to be approaching workable solution. Productive studies are already evident in this area where aspects of metabolism, enzymology,membrane transport processes are already underway with respect to mechanisms of drug action. In vitro and in vivo model systems for possible immunotherapeutic approaches to enhanced drug efficacy for malignant disease are also underway.

Particular effort with the clinically active agent duanomycin has elucidated many of the methodologic problems entailed in its study. However, the development of new fluorimetric and chromatographic techniques has permitted preliminary evaluation of its serum protein binding, tissue distribution, and potential approaches to modification of its considerable clinical toxicity. Studies oriented to cell-membrane localized events have yielded for the first time a stable, pure preparation of platelet membranes which may be preserved in the frozen state for subsequent study. Of interest has been the demonstration of direct inhibitory effects of duanomycin on platelet Na-K ATP-ase, an observation which therefore relates hematologic toxicity to a mechanism separate from megakaryocyte injury and broadens considerations on the mechanism of action of the drug. This enzyme-transport system is also under study from the point of view of steriodal hormone influence and may result in understanding mechanisms of cellular proliferation.

In immunology there has been increasing definition of the critical tissue culture characteristics which are required for reproducible evaluation of in vitro human and mouse normal leukocyte-tumor cell interactions. In particular cell concentrations has been found to have profound influence. Drug modification of such interactions and others of a similar immunologic nature are underway using l-amantadine as an initial test substance, in view of its presumed membrane-localized site of action.

Such studies along with others in earlier stages of progress attest to the vigor of the laboratory in the few months since its inception.

Serial No. NCI- 6010
1. Balto. Cancer Res. Ctr.
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on Human Chorionic Gonadotropin

Previous Serial Number: None

Principal Investigator: Joseph W. Jarabak, M.D., Ph.D.

Other Investigators: Nicholas R. Bachur, M.D., Ph.D.

Cooperating Units: Medicine Service, Baltimore Cancer Research Center (providing urine from tumor patients)

Man Years (July 1, 1967 to June 30, 1968)

Total:	0.1 Year
Professionals:	0.1 Year
Other:	None

Project Description:

Objectives:

To purify chorionic gonadotropin from individual patients with trophoblastic tumors in order to study the biochemistry and chemistry of this hormone

Major Findings:

Studies just initiated

Significance to Bio-med. Research and Program of the Institute

Chorionic gonadotropin from neoplastic tissue is suspected of being different chemically from the normal hormone. It is important to clarify this point since it may lead to understanding the invasive characteristics of trophoblastic tumors.

Proposed Course:

To continue early studies

Honors and Awards: None

Publications: None

Serial No. NCI- 6011
1. Balto. Cancer Res. Ctr.
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Effect of Free Fatty Acids on Ion Transport

Previous Serial Number: None

Principal Investigator: Khalil Ahmed, Ph.D.

Other Investigators: Miss Elizabeth Ward

Cooperating Units: None

Man Years (July 1, 1967 - June 30, 1968)

Total:	0.4
Professional:	0.3
Other:	0.1

Project Description:

Objectives:

In recent years there has been considerable interest in the study of effects of free fatty acids on the metabolic activity of tissues. The present investigation is undertaken to explore the effects of long chain free fatty acids on transport. As a first step, use is made of the model system referred to as the cation-activated ATP-ase of cell membranes and the effects of free fatty acids on the activity of this enzyme system are investigated.

Methods Employed:

Rat brain microsomal lipoprotein is isolated. This has a high Na-K activated ATP-ase activity. The system shows at least ten-fold increase in the ATP-ase activity when compared with Mg-activated component. Breakdown of ATP is studied by analysing the Pi liberated in the presence of enzyme system.

Major Findings:

Long chain fatty acids at relatively low concentrations inhibit cation-activated ATP-ase. The inhibitory effect increases with chain length. These effects are not related to MG binding and are reversed by changing ion concentrations in the reaction. The results tend to rule out the possibility that surface activity is the major cause of these effects. There appears to be a similarity in the action of these acids and oubain.

Significance:

The study of inter-relationships of inhibitor action on transport mechanisms is essential in understanding the action of many drugs.

Proposed Course of Study:

Structure-activity relationship of fatty acid to ATP-ase activity is now under investigation.

Honors and Awards: None

Publications: None

Serial No. NCI- 6012
1. Balto. Cancer Res. Ctr.
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Pharmacology of Daunomycin: Physical Chemical Properties of Daunomycin

Previous Serial Number: None

Principal Investigator: Mrs. Ann L. Liu
Nicholas R. Bachur, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years (July 1, 1967 to June 30, 1968)

Total:	0.6 Year
Professionals:	0.1 Year
Other:	0.5 Year

Project Description:

Objectives:

The need for adequate methodology for pharmacologic and biochemical studies of daunomycin and the unstable nature of daunomycin led to a study of the physical chemical characteristics of the drug and to the development of several methods. This is of importance in being able to separate metabolic products of daunomycin from degradative artifacts.

Methods and Observations:

Daunomycin and rubidomycin were obtained from the Drug Development Branch and were used to evaluate various paper, thin layer, and column chromatographic separation procedures. The quantification of daunomycin in tissue extracts and excretory products was developed through the use of both flurometric and spectrophotometric analysis. These methods are presently being used in studies on tissue distribution and biochemical properties of daunomycin.

It was observed that daunomycin could be handled with ease in the cold and in the dark but that it was very unstable in the presence of heat or visible light and that exposure to most activated surfaces tend to increase the breakdown of the drug.

Quantum efficiency of the fluorometric response of daunomycin in various solvents is variable and has been studied especially in relation to the quantification of drug from tissue extracts. Two standard techniques for fluorometric analysis of daunomycin are presently used.

Significance to Bio-med. Research and Program of the Institute

Understanding the fundamental properties of this extensively used drug is of obvious importance. Since little published work is available concerning this area , the past and future studies we have conducted will be of significant use.

Proposed course:

This project will be continued since several needs are not satisfied.

Honors and Awards: None

Publications: None

Serial No. NCI- 6013
1. Balto. Cancer Res. Ctr.
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Action of drugs on Platelet Membranes

Previous Serial Number: None

Principal Investigator: Donald E. Gutfreund, M.D.
Khalil Ahmed, Ph.D.
Nicholas R. Bachur, M.D., Ph.D.

Other Investigators: Miss Elizabeth F. Ward

Cooperating Units: None

Man Years (July 1, 1967 to June 30, 1968)

Total:	1 Year
Professionals:	0.8 Year
Other:	0.2 Year

Project Description:

Objectives:

This project is being developed from two points of view, firstly to study general aspects of platelet biochemistry (esp. phosphate metabolism) in health and disease and secondly to investigate the action of various drugs on platelet membranes. As a model cation activated ATP-ase was chosen to study the latter phenomenon.

Methods Employed:

Platelets are separated from contaminating erythrocytes and extensively washed. After osmotic rupture, fractionation is attempted by differential centrifugation or by using density gradients.

Major findings:

The presence of cation-activated ATP-ase in platelets has been demonstrated and its kinetic properties are being studied. The enzyme system is inhibited by ouabain at very low concentrations. Daunomycin also appears to inhibit the enzyme system but at relatively higher concentration. The nature of this action is being investigated.

Significance to Bio-med. Research and Program of the Institute

Platelet membranes have not been studied much in the past. The present work may throw light on relationship between coagulating activity and ATP-ase activity of these membranes.

Proposed course:

Platelet membranes will be isolated in relatively pure form and the action of drugs such as used in cancer chemotherapy or drugs which affect platelet aggregation will be studied.

Honors and Awards: None

Publications: None

Serial No. NCI- 6014
1. Balto. Cancer Res. Ctr.
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on Dihydrofolate Reductase from Human Trophoblastic Tissue

Previous Serial Number: None

Principal Investigator: Joseph W. Jarabak, M.D., Ph.D.

Other Investigators: Nicholas R. Bachur, M.D., Ph.D.

Cooperating Units: Medicine Service, Baltimore Cancer Research Center (providing malignant trophoblastic tissue)

Man Years (July 1, 1967 to June 30, 1968)

Total:	0.3 Year
Professionals:	0.3 Year
Other:	None

Project Description:

Objectives:

To purify dihydrofolate reductase from normal and malignant trophoblastic tissues and to study the effect of various agents on this enzyme

Major findings:

Studies just initiated

Significance to Bio-med. Research and Program of the Institute

An understanding of the resistance of some tumors to chemotherapeutic attack is necessary. This is especially critical in those tumors which seem to acquire resistance. The study of specific drug inhibited steps in resistant and non-resistant tumors may effect some understanding.

Proposed Course:

To continue early studies

Honors and Awards: None

Publications: None

Serial No. NCI- 6015
1. Balto. Cancer Res. Ctr.
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Cardiovascular Pharmacology of Daunomycin

Previous Serial Number: None

Principal Investigator: Jerrold G. Bernstein, M.D.
W. Leigh Thompson, Jr., M.D., Ph.D.

Other Investigators: Nicholas R. Bachur, M.D., Ph.D.
James C. Cradock

Cooperating Units: Medicine Service, Baltimore Cancer Research Center

Man Years (July 1, 1967 to June 30, 1968)

Total:	0.85 Year
Professionals:	0.75 Year
Other:	0.1 Year

Project Description:

Objectives:

To investigate the cardiovascular pharmacology of Daunomycin in animals in an attempt to understand the mechanism of the cardiotoxicity of this drug in man.

Methods Employed:

Early studies on the cardiovascular pharmacology of Daunomycin in the rabbit indicated a direct myocardial toxicity of the drug after prolonged treatment. This study was amplified in the following fashion: Atropinized, pentobarbital anesthetized dogs of either sex weighing approximately 10 kg. were used for these studies. Surgery was done under aseptic conditions. Animals were set up to record arterial blood pressure, myocardial contractile force (Walton-Brodie strain gauge arch) and electrocardiogram. Temperature was monitored and injections were made via a femoral vein catheter. Dose response curves for norepinephrine and isoproterenol were done prior to Daunomycin administration and daily for 4 days following Daunomycin, so that in their group each animal served as his own control. Also norepinephrine and isoproterenol dose response curves were done in animals over a 5 day period when no Daunomycin was given. Dose response curves were plotted in the form of rectangular hyperbolar and an IBM 7094 computer was used for data analyses.

Major Findings:

1. Daunomycin produces an immediate and prolonged bradycardia when given i.v. to dogs in a dose of 5 mg./kg.

2. Daunomycin seems to increase the incidence of premature ventricular contractions following isoproterenol and norepinephrine.

3. The contractile force response of the heart to isoproterenol and norepinephrine is modified by Daunomycin pretreatment. From 1 to 4 days after Daunomycin, the increase in contractile force produced by isoproterenol and norepinephrine is greater at lower doses but less at higher doses, while there is significant diminution in the mean maximal response of contractile force after Daunomycin.

4. No significant changes in heart rate or blood pressure responses to isoproterenol or norepinephrine were observed following Daunomycin treatment.

Significance to Bio-med. Research and Program of the Institute

This study serves to elucidate the mechanism of the clinical cardio-respiratory symptoms which have been observed in some patients treated with Daunomycin

Proposed Course:

The project will be terminated.

Honors and Awards: None

Publications: None

Serial No. NCI-6016
1. Balto. Cancer Res. Ctr.
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Tissue Distribution of Daunomycin

Previous Serial Number: None

Principal Investigator: Jerrold G. Bernstein, M.D.
Nicholas R. Bachur, M.D., Ph.D.

Other Investigators: Mrs. Ann L. Liu

Cooperating Units: None

Man Years (July 1, 1967 to June 30, 1968)

Total:	0.85 Year
Professionals:	0.75 Year
Other:	0.1 Year

Project Description:

Objectives:

To study the pattern of tissue distribution of the anti neoplastic agent, Daunomycin, in the adult mouse following intraperitoneal and intravenous injection.

Methods Employed:

Adult Swiss random bred male mice weighing approximately 25 grams were used for these studies. Animals were given Daunomycin (15 mg./kg.) either i.p. or i.v. via the tail vein. They were killed at various intervals following Daunomycin. Samples of the following tissues were removed and weighed (maximum tissue sample= 250 mg.):Brain, heart, lung, liver, spleen, kidney, skeletal muscle, and bile. Each tissue was then extracted.. The extracts were centrifuged and the supernatant assayed using an Aminco Microflourimeter, with an exciting wavelength of 470 nanometers. Flourescence was measured at 550 nanometers. The concentration of Daunomycin per unit weight of tissue was then calculated using a standard curve which was constructed for each day's experiment.

Major findings:

A simple reproducible method for extraction.

1. Recovery of approximately 90% could be obtained and analysis of Daunomycin was devised.

2. Initial levels of Daunomycin were present in all tissues examined on the first day, and slowly decayed thereafter.

3. The highest levels were present in the spleen.

4. Persistence of Daunomycin in the heart is of particular interest. Since the drug could be found in the heart tissue for up to 4 weeks following intravenous administration.

Significance to Bio-med. Research and Program of the Institute

The method of Daunomycin assay developed for use in this study may have usefulness in further clinical and laboratory studies on the metabolism of this compound. Furthermore the pattern of tissue distribution may have clinical applicability in terms of the further clinical use of this compound. Finally the persistence of daunomycin in myocardial tissue may be of significance in light of the cardiac toxicity observed clinically with this compound.

Proposed course:

The distribution studies will be continued and hopefully completed while amplification of the project into metabolic effect of Daunomycin and metabolism of Daunomycin will be started.

Honors and Awards: none

Publicatons: none

Serial No. NCI- 6017
1. Balto. Cancer Res. Ctr.
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Action of Androgens on prostatic ATP-ase

Previous Serial Number: None

Principal Investigator: Khalil Ahmed, Ph.D.

Other Investigators: H. Guy Williams-Ashman, Ph.D.

Cooperating Units: Department of Pharmacology & Experimental Therapeutics
The Johns Hopkins University Medical School
Baltimore, Maryland

Man Years (July 1, 1967 to June 30, 1968)

Total:	0.4 Year
Professionals:	0.4 Year
Other:	None

Project Description:

Objectives:

A study is made of the cation-activated ATP-ase system of rat prostate with a view to study the action of testosterone on this enzyme system in this tissue.

Methods Employed:

Method for preparing this enzyme system from rat prostate has been developed. And the enzyme system is isolated as a lipoprotein.

Major findings:

Prior to undertaking a study of the effect of testosterone, the basic properties of the enzyme system had to be understood. The kinetics of Mg,Na, K and ATP interaction were studied. Sensitivity to ouabain was determined. No effect of testosterone could be demonstrated in vitro.

Significance to Bio-med. Research and Program of the Institute

Relationship of this enzyme system to secretion by prostate and its control by androgens is of basic interest.

Proposed Course:

Experiments are underway to investigate the effects of testosterone in vivo using the cation-activated ATP-ase as a model for transport system.

Honors and awards: None

Publications: None

Serial No. NCI- 6018
1. Balto. Cancer Res. Ctr.
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Pharmacologic studies of Daunomycin: Binding of Daunomycin to Plasma Proteins

Previous Serial Number: None

Principal Investigator: Morton R. Axelrod, M.D.
Nicholas R. Bachur, M.D., Ph.D.
Technical Assistance: Miss Marta A. Kaber

Other Investigators: Joseph W. Jarabak, M.D., Ph.D.

Cooperating Units: None

Man Years (July 1, 1967 to June 30, 1968)

Total:	1.1 Year
Professional:	0.7 Year
Other:	0.4 Year

Project Description:

Objectives:

Pharmacologic properties of the cancer chemotherapeutic drug, Daunomycin, have been described in some detail but little correlation between some of the toxic phenomena and the proposed mechanism of action of the drug have been made. It has been stated that Daunomycin interacts primarily with nucleic acids of cells and has no observable interaction with proteins. We have undertaken a study to resolve a theoretical view of this drug's properties and the previously described experiments.

Methods and Observations:

Serum and plasma from normal individuals was used as protein source in initial studies. Simple dialysis, reverse dialysis, and infinite dialysis of daunomycin in presence of serum or plasma proteins indicated a binding of the drug to some non-dialyzable material. Electrophoresis of the plasma or serum proteins indicated the binding to be very weak and no direct relationship or quantification could be made.

Use of the Hummel and Dreyer technique with isolated plasma proteins from commercial sources has indicated binding of Daunomycin to several plasma proteins. The characteristics and specificity of this binding are presently being studied.

Significance to Bio-Med. Research and Program of the Institute

To understand the action of Daunomycin enough must be known of its biochemical pharmacology. This project is directed to elucidate some of this area.

Proposed Course:

The characteristics of Daunomycin interaction with proteins will be continued in order to use this information in the study of the mechanism of action of Daunomycin.

Honors and Awards: None

Publications: None

Serial No. NCI- 6021
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Mechanism of Oxygen Incorporation into Foreign Compounds

Previous Serial Number: NHI-323

Principal Investigator: Jordan L. Holtzman, M.D., Ph.D.

Other Investigators: James R. Gillette, Ph.D.
G. William A. Milne, Ph.D.
Peter Goldman, M.D.

Cooperating Units: Section on Drug Enzyme Interaction, Laboratory of Chemical Pharmacology, NHI

Section on Organic Chemistry, Laboratory of Metabolism, NHI

Section on Human Biochemical Genetics, Arthritis and Rheumatism Branch, NIAMD

Man Years (July 1, 1967 to June 30, 1968)

Total:	0.6 Year
Professionals:	0.6 Year
Other:	0 Year

Project Description:

Objectives:

The majority of drugs metabolized in the body as well as many of the reactions important in the metabolism of steroids in the mammalian organism involve enzymes which require molecular oxygen and NADPH. Because of the importance of the enzymes in the metabolism of drugs, and steroids and their probable significance in the activation of many carcinogenic agents to form the proximal carcinogen, it is of interest to examine the mechanism of these reactions. It is of further interest to compare these reactions to the bacterial dioxygenases to see whether the latter might provide a convenient model for the mammalian system.

Methods employed:

Standard Biochemical techniques were employed. Mass spectrometry was done by Dr. Milne on an L.K.B. 9000 combination mass spectrometer and on an A.E.I. MS-9 high resolution mass spectrometer. The bacterial cells were furnished by Dr. Goldman.

Major findings:

1. Further studies on the formation of 1,2 dihydro-1,2 dihydroxynaphthalene. We have previously shown that a single atom of molecular oxygen is incorporated into the hydroxyl groups of 1,2 dihydro-1,2 dihydroxynaphthalene (DHDIOL). We have now shown that this oxygen is incorporated into the number 1 position of the naphthalene. When DHDIOL formed in an atmosphere of $18\text{-}O_2$ was subjected to acid hydrolysis, the 1-naphthol formed contained 18-0 while the 2-naphthol did not. The DHDIOL was found to have an optical rotation of $[\alpha]^{25}D+412°$ ($C0.66$, $CHCl_3$). NMR data on a 100 Mc Varian spectrometer showed that the hydroxyl groups lay in a quasi-equatorial transconfiguration. From these data we concluded that the "activated" oxygen attacks the naphthalene stereo specifically in the number 1 position and forms an epoxide. The epoxide is then hydrolyzed either by the attack on the number 2 position by an OH^- ion to give DHDIOL or alternatively loses a proton and rearranges to form 1-naphthol, the other major metabolite.

2. The Formation of 3-fluorocatachol and catachol from 2-fluorobenzoic acid. A pseudomonas has been found which can utilize 2-fluorobenzoic acid as its sole source of carbon. This organism first converts the acid to either 3-fluorocatachol or catachol in the presence of molecular oxygen through a dioxygenase and then adds a second molecule of oxygen through a second enzyme, pyrocatachase. We have suspended these organisms in a buffer containing the acid with an atmosphere containing 50% of the oxygen as $18\text{-}O_2$. The reaction was stopped when the level of catachol had begun to decline. The catachol was then extracted and the extract dried. The mixture of catachol and 3-fluorocatachol was resolved on a L.K.B. 9000 combination gas chromatograph-mass spectrometer and the masses of the principle ions determined by rapid scans. These data indicated that the two oxygens added to both catachols were added as a single molecule and not in sequence.

Significance to Bio-med. Research and Program of the Institute

These studies should provide a better understanding of the factors which are important in determining the mechanisms of drug metabolism and carcinogenesis. And to further allow us to predict the metabolic pathways of a new drug or the carcinogenic potential of some foreign substance.

Proposed course:

The incorporation of oxygen-18 into a number of other pathways will be studied to further elucidate the fine details of the above mechanism.

Honors and awards: None

Publications: Holtzman, J.L., Gillette, J.R., and Milne, G.W.A.: The Incorporation of 18-0 into Naphthalene in the Enzymatic Formation of 1,2-Dihydronaphthalene 1,2-diol. J. Biol. Chem. 242: 4386-4387, 1967.

Holtzman, J.L., Gillette, J.R., and Milne, G.W.A.: The Metabolic Products of Naphthalene in Mammalian Systems. J. Amer. Chem. Soc. 89: 6341-6344, 1967.

Serial No. NCI- 6022
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Effect of Pretreatment with Phenobarbital and other Agents on the Metabolic Pathways of the Microsomal System

Previous Serial Number: NHI-319

Principal Investigator: Jordan L. Holtzman, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years (July 1, 1967 to June 30, 1968)

Total:	0.4 Year
Professionals:	0.4 Year
Other:	0 Year

Project Description:

Objectives:

Pretreatment of rats with phenobarbital and many other foreign compounds is known to enhance not only the oxidation and reduction of drugs by liver microsomes but also the metabolism of normally occurring substances. Among the metabolic pathways which have been shown to be enhanced are the hydroxylation of steroids to the more polar metabolites, the oxidation of glucose to pentoses and ascorbic acid, and the elimination of bilirubin into the bile. Isolated liver enzymes as glucose-6-phosphatase, UDPG transferase, and glucose-6-phosphate dehydrogenase as well as the more complex coupling in the mitochondrian of oxidation to the formation of ATP have also been reported to be enhanced by the administration of phenobarbital. The purpose of these studies is to elucidate the interrelationships of the various biochemical changes caused by phenobarbital.

Methods Employed:

Standard biochemical methods have been used.

Major findings:

1. Microsomal elements. In previous work I have demonstrated that the increase in microsomal phospholipid after the administration of phenobarbital to either male or female rats is due primarily to a decrease in the rate of destruction of the phospholipid and not to an enhanced synthesis. Other workers have suggested that a similar mechanism may be important in the observed increase in microsomal protein. I have taken young, adult male and female rats and administered 80 mg./kg./day i.p. of phenobarbital or an equivalent volume of saline for 20 days. On the 10th day I gave them 14-C (u.l.) L-glutamic acid and sacrificed animals at doubling time intervals from 45 min. to 196 hrs. The specific activity of the labeled protein from the control and treated animals were almost identical at all time intervals. This would indicate that, unlike its effect on phospholipid, phenobarbital does not cause a decrease in the catabolism of microsomal protein, but rather must cause an increase in synthesis. A further interesting observation was that the decay curve did not follow simple first order kinetics, but rather is better described as the sum of 2 or more first order curves. This would suggest that the elements which make up the microsomal fraction, whether they be primarily the endoplasmic reticulum from the hepatocytes or a mixture of this with other organelles from the hepatocytes or the reticulo-enodthelial cells, do not turn over as a single body. If the mass of the microsomes and their associated activity is from the endoplasmic reticulum of the hepatocytes, this would be evidence to indicate that the membrane does not turn over as little packets, but rather as individual molecules.

2. Purine and Pyrimidine Metabolism. The addition of orotic acid to the diet for six days causes a 30-50% reduction in aniline hydroxylation and a 10-20% decrease in N-demethylation of ethylmorphine by the 9000 g supernatant fraction from the livers of male, but not female, rats. This depression could be, at least partially, reversed by the addition of adenine sulfate to the diet. When the animals are maintained for 12 days on this diet, the enzymes begin to return to their normal levels. The effect is associated with the microsomal fraction and not with the supernatant, and in fact the supernatant from the treated animals seems to enhance the enzyme activity. In both male and female rats orotic acid had no effect on the induction by phenobarbital of these enzymes.

Significance to Bio-med. Research and Program of the Institute

These studies should lead to a better understanding of the effects of the chronic administration of drugs on liver function and the mechanisms which regulate the level of the microsomal enzymes.

Proposed course:

The incorporation of amino acids into protein will be studied under the previously described conditions. Further studies on the effect of other agents on the levels of the drug-metabolizing enzymes will also be examined.

Honors and Awards: None

Publications: None

Serial No. NCI- 6019
1. Balto. Cancer Res. Ctr.
2. Immunology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Search for the presence of tumor specific antigens on human neoplastic cells.

Previous Serial Number: None

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: Allen Bredt, M.D.

Cooperating Units: None

Man Years (July 1, 1967 to June 30, 1968)

Total:	1 Year
Professionals:	2/3 Year
Other:	1/3 Year

Project Description:

Objectives:

To search for and define the presence of tumor specific antigens in leukemic cells in man.

Methods Employed:

Short term tissue culture involving cell-cell interaction was employed. The presence of qualitative or quantitative differences in membrane antigens between neoplastic and syngeneic normal cell populations was determined through DNA synthesis experiments in which increased synthesis of DNA has been shown to reflect antigenic differences between lymphocyte populations. A patient with acute lymphocyte leukemia possessing a monozygote twin was studied in this system. DNA synthesis within cells of a third normal individual mixed in culture with cells of the leukemic twin and cells of the normal twin was used as a measure of differing cell antigenicity.

Major Findings:

1. A qualitative or quantitative antigenic dissimilarity exists between the peripheral white cells of the leukemic patient and those of his identical twin.

Proposed Course:

1. Further delineation of the antigenic difference between the patients leukemic cells and those of his brother.

2. To continue similar experimentation in other patients who possess monozygotic twins.

3. Production of highly specific antibody and sensitized cells against human leukemic cells demonstrated to differ antigenically from normal syngeneic cells.

4. The utilization of specifically sensitized cells and/or specific antibody as a experimental therapeutic modality for the treatment of leukemia.

Honors and Awards: None

Publications: None

Serial No. NCI-6020
1. Balto. Cancer Res. Ctr.
2. Immunology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Mechanisms of human lymphocyte reactivity to non-self

Previous Serial Number: None

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: Allen Bredt, M.D.

Cooperating Units: None

Man Years (July 1, 1967-June 30, 1968)

Total:	1	Year
Professionals:	2/3	Year
Other:	1/3	Year

Project Description:

Objectives:

To define basic mechanisms of human cell reactivity to non-self and to differentiate the mechanism of reactivity of normal human lymphocytes to allogeneic lymphocytes from that of mitogenic substances whose mode of stimulation of normal human lymphocytes may not be related to the immunologic potential of this cell.

Methods Employed:

Short term tissue culture techniques, and measurement of tritiated thymidine incorporation into DNA.

Measurement of cell reactivity and non-reactivity with 1-adamantanamine hydrochloride.

Major Findings:

1. The mechanism of reactivity of a normal human lymphocytes to allogeneic lymphocytes differs from that mitogenic activity initiated by phytohemagglutinin.

2. 1-adamantanamine hydrochloride can specifically block the mitogenic manifestation of the recognition of allogeneic lymphocytes by normal human lymphocytes in vitro.

3. 1-adamantanamine hydrochloride enhances phytohemagglutinin stimulation of normal human lymphocytes at the same dosage it blocks reactivity to allogeneic lymphocytes.

Proposed Course:

1. Define site and mechanisms of blockage of 1-adamantanamine hydrochloride of normal human lymphocyte recognition of non-self.

2. Evaluate the *in vivo* potential of 1-adamantanamine hydrochloride to elicit specific immunologic unresponsiveness in the experimental animal.

Honors and Awards: None

Publications: None

ANNUAL REPORT

OFFICE OF ASSOCIATE SCIENTIFIC DIRECTOR FOR EXPERIMENTAL THERAPEUTICS

LABORATORY OF CHEMICAL PHARMACOLOGY

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

July 1, 1967 -- June 30, 1968

The pharmacological disposition of the following compounds has been studied this year: Cyclohexylchloroethyl nitrosourea (CCNU), cyclophosphamide, anthramycin methyl ester, camptothecin, asparaginase, related congeners of asparagine, hydroxyurea, and guanazole. One major effort was to identify the metabolic products of CCNU. It is thought that the formation of a isothionate is the step which causes not only toxicity, but activity. By using CCNU with either the cyclohexyl or ethylene moiety labeled with C^{14}, information was obtained concerning the breakdown of the product. The ethylene half of the CCNU has no difficulty entering brain or cerebrospinal fluid while the cyclohexyl half, by virtue of extensive plasma protein binding, appears in cerebrospinal fluid to a lesser extent.

Interest in the microsomal drug metabolizing enzyme systems focused on potential metabolism of cyclophosphamide. Cyclophosphamide _per se_ is inactive and must be metabolized by hepatic microsomal enzymes to the active compound which may be either cytoxalamine, cytoxalalcohol or possibly other related compounds. Inhibition of metabolism of cyclophosphamide in the mouse yields a greater toxicity with an earlier onset of death suggesting that cytoxan _per se_ has whole animal toxicity in the mouse. Collaborative studies are underway to investigate cyclophosphamide metabolism in patients. Parallel investigation with the toxicity and therapeutic activity of cytoxalalcohol and cytoxalamine have shown that the alcohol is fully as active as cyclophosphamide whereas the amine compound at quite high doses is inactive. Because of the possibility that man may not make adequate amounts of the active metabolite, we are preparing for clinical trial of cytoxalalcohol. Preliminary studies have been undertaken concerning the pharmacological disposition of the plant alkaloid, camptothecin, and the simple synthetic compound guanazole.

The comparative pharmacology of biliary excretion has been studied. Of interest is that pretreatment with phenobarbital, which enhances microsomal enzyme metabolism, also enhances biliary excretion of certain compounds. These studies in rats will be extended to other species.

The work with asparaginase and asparagine analogs has yielded the information that S carbamyl-l-cystine is active in L1210 and that L-asparaginase is teratogenic in the rabbit. Preliminary pharmacologic studies with tetrahydrohomo folic acid are underway.

In conjunction with the pharmacological activities in the intramural program, there have been frequent meetings in the past year of the pharmacology committee composed of intramural scientists and contractors involved in similar studies. Highlights of recent meetings have included the description of cyclophosphamide metabolism by Mellette of Southern Research Institute; studies on the disposition of methyl mercaptopurine riboside, and the imidazolecarboxymid compounds by Loo and Frei of M.D. Anderson; studies of trifluorodeoxyuridine as well as 6-Mercaptopurine and fluorouracil by Rogers at Arthur D. Little. The purpose of this committee is to insure that all compounds are studied and that unnecessary duplication does not occur as well as to provide for the exchange of important information. The base of pharmacologic information on the important anticancer drugs is of an adequate size that we can begin to relate plasma and tissue concentration time relationship to tumor cell kill and host toxicity. This will be a major effort for next year.

Studies on the mechanism of action of selected anticancer agents has continued. Anthramycin appears to bind only to helical DNA and the binding appears to be of a covalent nature. The role of hydroxyurea in nucleotide reductase systems is being elucidated. The complex methods needed for these studies are being adapted to mammalian cells such as L1210 grown in tissue culture.

Studies on the pharmacology of brain tumors and in the normal blood-brain and cerebrospinal fluid barriers progressed in two directions. Model systems for brain tumors in animals are being developed. Gliomas and ependymoblastomas in rodents are being studied for their response to chemotherapeutic agents. The ependymoblastoma is responsive to the nitrosoureas whether planted subcutaneously or intracranially. Intracranially implanted ependymoblastoma, however, that does not respond to cyclophosphamide. In studies of the normal blood-brain barrier we have clearly demonstrated for the first time the manner in which the cerebrospinal fluid can serve as a sink for compounds present in the extracellular space in the brain. Since the central nervous system lacks lymphatics, the usual mechanism for removing proteins and other lipid insoluble molecules. It has been suggested in the past that CSF functions in lieu of a lymphatic system in the brain. This hypothesis was tested by looking for concentration gradients of lipid insoluble substances within brain tissue. Gradients have been demonstrated in two vertebrate species and thus experimental proof of such a sink effect of the CSF is at hand. These concentration gradients indicate a new diffusion from brain into CSF of such lipid insoluble compounds as sulfanilic acid, mannitol, inulin and sucrose. This is a logical extention of the studies performed in this laboratory which first clearly showed the existence of a relatively large extracellular space in the brain and has now moved on to an explanation for the functions of such a space.

Preclinical toxicology of anticancer drugs has been pursued vigorously this year. Toxicology protocols have been redesigned to differentiate between studies performed early in the development of a compound and studies performed after a compound has been in man for variable periods of time. Type I Toxicology is a simple system designed to define, rapidly, the qualitative and quantitative toxicity of potential antitumor drugs. Type II Toxicology is a further elucidation of the qualitative and quantitative aspects of toxicity in other large animal species in dose schedules designed on the basis of experimental animal tumor studies. Type III Toxicology is a specialized combination or chronic toxicity study to be performed only on compounds which have extensive clinical trial.

Certain highlights of the preclinical toxicology program include the finding that NSC-13002, a dimethylaminoacridine, is a potent central nervous system convulsant and severely damages the pulmonary system. Two of the dogs receiving this compound developed pulmonary neoplastic changes including a focus of an anaplastic bronchogenic carcinoma. L-asparaginase, NSC-109,229, was shown to have significant hepatotoxicity in the rhesus monkey as evidenced by causing a reversible fatty liver and BSP retention.

A Type III Toxicology study of ortho,para'-DDD is completed. One of the most vexing toxic manifestation of cyclophosphamide in clinical use is alopecia. Usual animal species do not show alopecia after cyclophosphamide. In both the poodle dog and in sheep, the hair grows continuously as it does in human hair, but unlike the sporatic growth of the body hair of most animals. After cyclophosphamide, both the poodle dog and the sheep lose their hair and become bald as seen in man. This should prove to be a useful system in which to study the effect of such agents on hair growth.

There has been considerable effort in this laboratory on experimental therapeutics this past year. The problems of scheduling drugs in the treatment of tumors of varying generation times and volume doubling times obviously requires a mathematical approach. With the collaboration with Dr. Patlak, National Institute of Mental Health, a simple but apparently realistic mathematical model is being developed of the growth of solid tumors and the effect of anticancer agents on this growth. We hope that this will be useful in studies of both the laboratory animal and in clinical cancer chemotherapy.

The Brain Tumor Study Group consisting of a number of academic neurosurgeons dedicated to quantitative clinical trials of anticancer drugs in brain tumors is performing smoothly. The first protocol using mithramycin is well underway. Methods for randomizing patients and retrieving data are working well. This protocol should be completed within the year and discussions are now centered on other potential chemotherapeutic agents.

Serial No. NCI-3500
1. Laboratory of Chemical Pharmacology
2. Pharmacology Section
3. Bethesda, Maryland

NIH-PHS
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Penetration of Antitumor Drugs Into the Central Nervous System

Previous Serial Number: Same

Principal Investigators: Joseph D. Fenstermacher and David P. Rall

Other Investigators: Clifford S. Patlak, Michael D. Walker, James I. Ausman, William R. Shapiro, Victor A. Levin, Thomas Clancy, Milton W. Brightman, Thomas S. Reese, and Choh-luh Li

Cooperating Units: Biometrics Branch, NIMH; Section of Neurological Surgery, Baltimore Cancer Research Center; Laboratory of Neuropathology and Neuroanatomical Sciences, NINDB; and Surgical Neurology Branch, NINDB.

Man Years

Total:	5
Professional:	3
Other:	2

Project Description:

Objectives:

To quantify the transport of various materials of physiological and pharmacological importance between blood, brain, and cerebrospinal fluid (CSF). It is the purpose of such studies to characterize the transport properties of the blood-brain-CSF system and to aid the development of chemical methods for obtaining more effective central nervous system (CNS) concentrations of antitumor drugs.

Methods Employed:

The method of ventriculo-cisternal perfusion of various radioactive compounds has been utilized in anesthetized dogs, cats, and rabbits. Both isotopic and histological analysis of the brain are employed. In addition studies of brain and CSF uptake and distribution of various radioactive materials injected intravenously have been examined. These studies have been done in normal, hypothermic (15°C), and tumor bearing animals.

Major Findings:

Intraventricular perfusion for the treatment of malignant human brain tumors has been continued, although at a reduced pace because of the limitation of bed space. The intraventricular toxicity of cytosine arabinoside and 8-azaguanine are being evaluated in dogs and monkeys.

A major effort to develop a system within primates in which one could study an intracranial neoplasm continues. Studies of the distribution of well known as well as more esoteric compounds in normal brain cerebrospinal fluid versus transplantable intracranial neoplasms are being carried out. The principal effort has been within the transplanted semian hepatoma in the brain of newborn or juvenile monkeys. This is described in project (NCI-3509) and appears to have the possibility of becoming a reproducible system.

We studied the basic pharmacology of tritiated mithramycin. This has not been done only in terms of its distribution throughout the body, plasma half life, and modes of excretion, but its comparative distribution within the brain and transplanted brain tumor. This drug is considered of importance as it has previously been reported to be effective in the treatment of human glioblastoma multiforme.

From our ventriculo-cisternal perfusion studies we are measuring the size of the CNS extracellular space (ECS), the regional variation in this space within the brain, and the effects of hypothermia and tumor growth upon this space. From these same experiments we are determining by radioisotopes and electron microscopy the adequacy of the ECS to serve as a pathway for the distribution of pharmacological agents and physiologically important materials from the blood and CSF to the brain tissue. With these methods we are examining the cellular uptake, the capillary permeability, and the cellular binding of various substances, also.

Studies have been made of the blood volumes and hematocrits of the various parts of the CNS in normal and tumor bearing animals.

A considerable portion of time has been devoted to purifying and characterizing C^{14}-and H^{3}-inulin, a substance generally used to measure the size of the extracellular space. Because of the impurities in this radioactive material serious question can be raised concerning its adequacy and use as such a marker. This would have considerable effect upon our work and the work of others. Gel filtration, dialysis, and diffusion separation have been used to purify and analyze this.

Measurements of the diffusion coeffiecients of many organic materials in water and tissue and the effects of temperature, crystal formation, and some histological fixatives upon this process have been performed.

An attempt is being made to isolate and perfuse the choroid plexus of the calf. So far the effort has met with reasonable success although viability and normal function of the excised plexus has not been unequivocally established. It is hoped that such a preparation will enable direct studies of blood to CSF exchange across the choroid plexus.

We have continued to pursue studies in the comparative physiology and pharmacology of blood-brain-CSF transport. We have obtained evidence in elasmobranch that the cerebrospinal fluid can serve as a "drain" or "sink" for various materials present in the brain, i.e. various materials may be removed from the brain by diffusion into the CSF. These studies were done at the Mount Desert Island Biological Laboratory, Maine, and the Lerner Marine Laboratory, Bimini, the Bahamas. Similar experiments were performed in dogs at NCI-LCP. Work is being initiated upon the transport of material between the CSF and spinal cord of lampreys. The peculiar advantage of this system is that it is avascular, enabling one to ignore the effects of blood-brain transport.

The Brain Tumor Chemotherapy Study Group has been actively functioning both as a research forum and a collaborative neurosurgical group for the evaluation of chemotherapy in malignant brain tumors. Patient and data accrual is proceeding satisfactorily and new collaborating members are being integrated into the working protocol program. The first protocol study of the group is a controlled study of Mithramycin in glioblastoma with comparative analysis of survival time of post operative patients, under the joint sponsorship of NCI and NINDB. The current state of brain tumor chemotherapy research was reviewed in detail by several study group members at a symposium on Brain Tumors under the auspices of the New York Academy of Sciences.

Significance to Bio-medical Research and the Program of the Institute:

Tumors, both primary and metastatic, frequently develop in the central nervous system. Little information is available concerning the penetration of antitumor drugs into the CNS or into neoplastic disease within the brain. Basic research on this aspect of the pharmacology of antitumor drugs is necessary for the rational use of these agents. Further, it becomes clear that the central nervous system acts in many ways as a typical cell and knowledge of the entry of drugs into the central nervous system parallels the entry of drugs into cells in general. The differences, however, between the central nervous system and other cells in general can and are being exploited to chemotherapeutic advantage.

In connection with this goal, we have also undertaken extensive studies of the transport of various physiological materials between blood, CNS, and CSF in normal and tumor bearing animals. An understanding of the basic features of this system should aid the development of effective dose schedules for various antitumor agents as well as to analyze why such drugs are or are not useful clinically for cancer chemotherapy.

The Brain Tumor Chemotherapy Study Group functions as an initial effort in a controlled prospective study of the palliative effect of chemotherapeutic agents on malignant brain tumors, attempting to extend the methods of other collaborative chemotherapy programs in other diseases to the virtually virgin area of malignant neoplasms of the central nervous system.

Proposed Course:

1. Continue the studies of the extracellular space and its function in normal, hypothermic, and tumor animals. Proceed to further analyze the transport of various materials including drugs between blood, brain cells, and cerebrospinal fluid in normal and tumor animals.

2. Further attempts to elucidate some of the basic pharmacological factors determining both how much, and how fast drugs get into the CNS and the spinal fluid from plasma. These include cerebral blood flow, state of thyroid function, etc.

3. Continued investigation of the comparative pharmacology of CNS and CSF. The technical ease with which the ventricular fluid, choroid plexus, and brain are obtained in elasmobranch make these species particularly valuable.

4. Continued investigation of the possibilities of the therapeutic potentiality of ventriculo-lumbar perfusion in patients with intracranial neoplasms.

5. Further work and the possibilities of establishing a uniform and transplantable brain tumor model in primates will be continued. The effects, distribution, and differences of various chemotherapeutic agents within these systems will be investigated.

6. Continue the work with the isolated-perfused choroid plexus.

7. The study group plans to expand its effort, aiming eventually at a comprehensive test system in human brain tumors, whereby new chemotherapeutic agents can be tested for their effect on primary neurological neoplasms.

Publications:

Rall, D. P. and Burger, J. W.: Some Aspects of Hepatic and Renal Excretion in Myxine. Amer. J. Physiol., 212:354-356, 1967.

Rubin, R. C., Henderson, E. S., Owens, E. S. and Rall, D. P.: Uptake of Methotrexate-^{3}H by Rabbit Kidney Slices. Cancer Res., 27:553-557, 1967.

Cserr, H. and Rall, D. P.: Short Communication. Regulation of Cerebrospinal Fluid [K+] in the Spiny Dogfish, Squalus Acanthias. Comp. Biochem. Physiol., 21:431-434, 1967.

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Rall, David P.: Pharmacologic Aspects of Selective Chemotherapy of Leukemia and Burkitt's Tumor. Combination Chemotherapy: Advertent and Inadvertent. Cancer Research 27 Part 1, 2650-2655, 1967.

Rall, David P.: Selective Aspects of Chemotherapy in Acute Leukemia and Burkitt's Tumor. Cancer, Vol. 21, No. 4, 575-579, 1968. In Press

Bearing, E. A., Jr., Rall, D. P., and Oppelt, W. W.: Hydrodynamics of the Cerebral Ventricular Fluid in the Shark. In Gilbert, P. W., Mathewson, R. F., and Rall, D. P. (Eds.): Sharks, Skates and Rays. Baltimore, Md., The Johns Hopkins Press, 1967, pp. 389-398.

Rall, David P.: Comparative Pharmacology and Cerebrospinal Fluid. In Cafruny, E. J., Cosmides, G. J., Rall, D. P., Schroeder, C. R. and Weiner, I. M. (Eds.): Proceedings of An International Symposium on Comparative Pharmacology. Bethesda, Maryland, Federation of American Societies for Experimental Biology, pp. 1020-1023, 1967.

Serial No. NCI-3505
1. Lab. of Chemical Pharmacology
2. Pharmacology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Chemical Carcinogenesis

Previous Serial Number: Same

Principal Investigators: Margaret G. Kelly, Roger W. O'Gara, and Vincent T. Oliverio

Other Investigators: None

Cooperating Units: Laboratory of Pathology, NCI; Serial No. NCI-526(a)

Man Years

Total:	1.5
Professional:	0.5
Others:	1.0

Objectives:

1. To determine the carcinogenic activity of chemicals to which the human population may be exposed, particularly drugs of interest in cancer chemotherapy.

2. To compare the susceptibility of newborn vs. adult animals.

3. To characterize and transplant such induced tumors as new tools for experimental cancer chemotherapy.

Methods Employed:

Usual biologic, pharmacologic, and histopathologic techniques.

Major Findings:

1. The carcinogenicity of N-isopropyl-α-(2-methylhydrazino)-p-toluamide (MIH) its in vivo degradation products, and a group of related hydrazines have been studied to obtain information as to whether MIH per se or one of its degradation products is responsible for its antitumor and carcinogenic actions. Studies with the degradation products suggest that they may contribute in large part to the pulmonary tumors induced in mice with MIH - this is especially true for the azo and the hydrazone compounds. However, only MIH and the azo compound are responsible for the induction of leukemia and for the toxicity.

2. The acute toxicity (mg/kg or mg/M^2) of a single intracerebral injection of 1-methyl-1-nitrosourea (MNU) was not significantly different in newborn Sprague-Dawley rats and in newborn General Purpose and CDF_1 mice. In newborn mice toxicity by the intracerebral or the subcutaneous route of administration was comparable. None of the rats or mice given an intracerebral injection of MNU as newborns showed gross signs or histologic evidence of tumors of the brain when adults and none of the mice given a subcutaneous injection of MNU at birth developed tumors at a later date at the site of injection. A few rats given MNU by the intracerebral route as newborns developed sarcoma of the kidney and one rat (14 weeks old) had a mammary adenocarcinoma when 14 weeks of age. However, leukemia was induced in 30-80% of mice and multiple pulmonary tumors in 80-100% after either an intracerebral or a subcutaneous injection of MNU at birth. The data suggests that MNU is rapidly absorbed from the brain and subcutaneous tissue into the systemic circulation with little loss of carcinogenic activity.

Significance to Bio-medical Research and the Program of the Institute:

1. Nitrosamines and nitroso compounds are possible carcinogenic materials in our environment. It is important to evaluate their potential hazard, both in the young and in the mature population.

2. Many drugs used in the chemotherapy of cancer are knownto be carcinogens. Few, however, have exhibited such strong carcinogenic activity as MIH, and none elicit such a wide spectrum of tumors in mice and rats. With the recent clinical use in MIH, it is essential to carefully delineate its carcinogenic potential.

Proposed Course:

The carcinogenicity of other agents used in chemotherapy and especially in cancer chemotherapy will be investigated.

Serial No. NCI-3506
1. Lab. of Chemical Pharmacology
2. Pharmacology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Pharmacology of Alkylating Agents and Other Antitumor Drugs

Previous Serial Number: Same

Principal Investigators: Margaret G. Kelly and Richard H. Adamson

Other Investigators: Dr. M. Terry McEnany, Immunology Branch, NCI

Cooperating Units: Immunology Branch, NCI

Man Years

Total:	0.9
Professional:	0.3
Others:	0.6

Objectives:

1. To study the pharmacology of antitumor drugs of current interests in clinical chemotherapy.

2. To evaluate the usefulness of new tumor systems in experimental chemotherapy.

3. To explore the possible usefulness of tumor-specific antigens on the growth of experimental tumors.

Methods Employed:

Usual pharmacologic, biochemical, immunologic, and histopathologic techniques.

Major Findings:

1. The lymphocytic leukemia induced in CDF_1 mice with MIH (N-isopropyl-*α*-(2-methylhydrazino)-*p*-toluamide has been carried in this strain as a transplanted tumor (SC and IP). This leukemia, like L1210, invades the thymus, lungs, lymph nodes, brain, spleen, kidney, liver, ovaries, and terminally gives a high total leukocyte count. Like L1210, the MIH-induced leukemia responds to therapy with cytoxan. Unlike L1210, however, the MIH-induced leukemia does not respond to chemotherapy with methotrexate. Vincristine, 6-MP, and prednisone also are ineffective in the treatment of the MIH leukemia and MIH (the inducing drug) causes a moderate (30-50%) increase in survival time.

The effect of cytoxan and several possible metabolites is being investigated against L1210 in vivo and in vitro. To date only cytoxal alcohol and cytoxan have antitumor effect against L1210 in vivo.

The effects of several antitumor antibiotics in different tumor systems is being assessed in order to find a system that is sensitive to them and one that might predict cross resistance to agents having the same presumed mechanism of action.

2. (With Dr. McEnany): Melanoma S91 and plasma cell tumor LPC have been used in attempts to influence the growth of these tumors by treating with "specific antigens" prepared by coupling the tumor cells with either rabbit gamma globulin or hemocyanine. Several schedules and routes of immunization and different antigen vehicles were used in three separate series of experiments using 2,000 mice. Using the conjugated tumor cell as the antigen, protection against the syngeneic tumor graft was not consistently demonstrated. A significant humoral response to the protein addative was detected in these animals, however. When foreign protein was conjugated to syngeneic splenocytes in the same fashion, treatment with this antigen led to prolonged host survival, in the case of LPC-1, and to delayed appearance of S91 tumor nodules.

Significance to Bio-medical Research and the Program of the Institute:

Careful evaluation of the effect of antitumor compounds in induced tumor systems should be made until sufficient data is available to judge their usefulness in screening compounds for ultimate clinical trial in the tumor patient.

Proposed Course:

1. Further comparisons will be made of the response of leukemia L1210 and the MIH-induced leukemia, and in addition with a leukemia recently induced in this laboratory with 1-methyl-1-nitrosourea to a spectrum of chemotherapeutic agents. As all three leukemias are lymphocytic neoplasms indistinguishable on common histopathologic examination, it should be interesting to note their similarities and differences in response to therapy. Furthermore, the fact that these leukemias are all carried in the same strain of mouse should make host toxicity a common factor in evaluating therapeutic effect.

2. Use of induced tumors (primary and transplanted) for chemotherapy will be continued.

Serial No. NCI-3507
1. Lab. of Chemical Pharmacology
2. Pharmacology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Preclinical Toxicology of Drugs Potentially Useful in Cancer Chemotherapy

Previous Serial Number: Same

Principal Investigator: Elton R. Homan

Other Investigators: Richard H. Adamson, Howard Bruckner, Philip S. Schein, Michael D. Walker

Cooperating Units: Hazleton Laboratories, Inc., Falls Church, Virginia; Mason Research Institute, Worcester, Massachusetts; and Pico Laboratories, Inc., San Antonio, Texas

Man Years

Total:	3
Professional:	2
Other:	1

Objectives:

The objectives of this project are: 1) to evaluate the toxicologic and pharmacologic properties of potential cancer chemotherapeutic agents in animals for the purpose of expediting clinical trial, and 2) to study the influence on drug toxicity of dosage scheduling, concomitant drug administration, and alterations of environmental variables in order to develop better predictive models of toxic responses and to optimize therapeutic effects.

Methods Employed:

The primary tool in these investigations is the dosage response relationship in experimental animals as reflected in pharmacologic parameters and in mortality. Conventional laboratory methods of drug administration and evaluation of effects in one or more animal species are used in many studies, although it is frequently necessary to develop specialized approaches where existing techniques are inadequate. Statistical analysis of results are performed wherever appropriate for the needs and capabilities of an experiment.

Major Findings:

1. Toxicology of Antitumor Agents Administered Intrathecally to Monkeys

Toxicologic evaluation of several chemotherapeutic agents has been conducted under the guidance of Dr. Walker following intrathecal administration to monkeys. The spectrum of toxic effects is considerably altered from that seen after usual routes of administration. Agents in various stages of investigation include cytosine arabinoside, methotrexate, l-phenyl-alanine, and l-asparaginase. Anorexia, weight loss, and depression are typical sublethal findings and probably represent nonspecific CNS toxicity. A high incidence of convulsions, frequently culminating in death has followed MTX administration. Attempts to prevent the convulsions associated with MTX by prior administration of dilantin or phenobarbital were not conspicuously successful.

Convulsions seen after cytosine arabinoside administration in early studies were absent in subsequent work in which the drug was buffered. Twice weekly doses of buffered cytosine arabinoside after a total of five administrations led to dose related weakness, hyperexcitability, flaccid paralysis and coma. Possible mild marrow depression was indicated also.

2. Toxicology of 5-Fluorouracil Administered to Dogs by Intravenous Infusion and Frequent Oral Administration

This project was initiated to compare the effects of similar doses of 5-FU administered to dogs by continuous intravenous infusion and by frequent oral dosing for comparable periods of time. Sublethal doses characteristically produced weight loss and SGOT and SGPT elevations, all of which were reversible. Convulsions, both during and after 5-FU administration, were seen frequently in both animals which survived and which died. No differences, qualitative or quantitative, could be detected between the routes or between infusion durations of 24 to 48 hours.

Since no useful information was anticipated from postmortem examination of survivors, it was decided to redose these animals. Dogs which had been previously treated were found to tolerate larger doses than naive dogs. The appearance of convulsions frequently jeopardized the completion of early experiments. Small doses of pentobarbital (6-12 mg/kg) broke the convulsions and increased survival. Pretreatment with dilantin or phenobarbital produced no similar effect.

In an experiment designed to test the protective effects of prior dosing with 5-FU, mice were given repeated 5-day intravenous courses every two weeks, each course larger than the previous. In this study, survival at each of several dose levels was increased in sequential trials. While this might represent simple selection, altered metabolism appears more likely.

3. Repeated oral administration of o, p'-DDD to rats and dogs

Results of daily oral dosing of rats and dogs for twenty eight days were found similar to published data from shorter term toxicity studies. Rats are relatively insensitive to this agent and exhibited only elevations in alkaline phosphatase activity at the high dose (300 mg/kg/day).

Dogs were, as in published literature, considerably more sensitive and exhibited sharp increases of alkaline phosphatase and SGOT activity and atrophy and lymphocytic infiltration of the adrenal cortex. The high dose level of 100 mg/kg led to mortalities and was decreased to 75 mg/kg/day. A novel and efficient method of obtaining jugular vein blood from the rat was developed and is being prepared for publication. One unexpected result of gas chromatographic studies is the apparent metabolic conversion of o, p'-DDD to p, p'-DDD. This anomalous change should be explored further.

4. Toxicity of o, p'-DDD to Pregnant Bitches and Their Progeny Following in utero Exposure

This experiment was conducted in response to an inquiry resulting from an analogous clinical situation. Two pregnant bitches were given fourteen daily oral doses of the adreno-corticolytic agent, o, p'-DDD, during the second trimester of pregnancy. Five days after the end of treatment, a hysterectomy and hemiadrenalectomy were performed on one bitch to provide material for histologic and chemical analysis; the other bitch was allowed to deliver at term.

Comparable tissue concentrations of DDD were found in the pups and bitches. However, although typical histologic changes were seen in the bitches, there was no evidence of adrenocortical histologic damage or functional insufficiency in the pups. Apparently only differentiated functional adrenocortical tissue is susceptible to o, p'-DDD toxicity.

5. Toxicology of Cytoxan with Special Reference to Hair Growth

Cytoxan is one of several chemotherapeutic agents which cause alopecia in patients. Conventional laboratory animal species fail to predict this effect although patchy hair loss has been noted on occasions. The availability of a suitable animal model would be useful in several respects: 1) prediction of agents with this type of liability, 2) the study of means of preventing or ameliorating alopecia, and 3) a means of performing cell kinetic studies on a readily accessible target tissue.

The development of complete hair loss in a poodle following the administration of several courses of cytoxan provided incentive for numerous subsequent studies. The following conclusions and observations have been derived: the demonstration of hair loss in the poodle is best achieved by large single doses; hair loss can be achieved at doses which produce only minimal hematologic and biochemical signs; hair loss is delayed about ten to fourteen days after dosing and is completely reversible by growth of new hair; the regional distribution over the body of susceptibility to hair loss is apparently positively correlated with hair length and local skin temperature and therefore presumably with rate of cell division in the follicular tissue; the phenomenon is apparently general to animals with continuously growing hair, as demonstrated by the poodle, sheep, and Angora rabbit. Studies in Peruvian guinea pigs are not yet adequate to permit comparison. Dosing of beagles confirmed earlier work which showed no similar toxicity, although one beagle in these studies exhibited "looseness" of the hair shafts.

Cytoxan has been found to be considerably less toxic to chicken than to mammalian species. The implications of this difference relative to metabolism bear consideration, and may be useful in the study of phylogeny of mechanisms of detoxication.

6. Studies on Dosage Scheduling and Drug Combinations

These studies, which may be considered an extension of similar work carried out during the previous year, have as their purpose both the evaluation of drug toxicity and the development of optimal methods of evaluation. Both empirical and rational approaches have been used in the design of experiments. A number of drug combinations and dosage regimens were employed and several significant interactions are apparent. One conspicuously absent is the so-called "priming effect" of methotrexate. It may very well be that special conditions are necessary for its demonstration, and there is tentative evidence that the condition of the intestinal flora may be critical through its effect on folate metabolism.

It is not yet possible to relate the observed interactions in terms of general principles, beyond the integration of dose with cell cycling, which itself is, so far as lethality studies are concerned, inferential.

Efficiency of experimental design has been shown to be an important consideration since the necessary number of animals is an exponential function of the number of input variables. At this stage, many of the studies require confirmation by repetition. Since the facilities and time available are limited, these studies are restricted to the level of about one study every three to four weeks.

7. Methodologic Studies on the Evaluation of Liver Function

A number of investigations have been undertaken to develop a suitable screen of hepatotoxicity of antitumor agents. Since for several reasons it would be convenient to use mice, tests adaptable to this species were sought. The hexobarbital sleeping time is an accepted means of evaluating microsomal oxidative systems and since there appears to be some parallelism in the alteration of various drug metabolic pathways following the administration of hepatotoxic agents, it would appear to have broad utility. Sleeping times have been measured following administration of a number of agents and a satisfactory standardized routine is being developed. The method is not without limitations, however. One aspect of this, which has not been noted in published material is the necessity of performing the test under uniform noise conditions. Since the mice are immobile but hyperexcitable during certain stages of anesthesia, a sharp noise can precipitate a chaotic termination of the test. Rigorous adherance to established endpoint criteria is necessary, and the endpoint must be so defined as to obviate the problem of malingering.

One observation in these studies particularly bears further exploitation. Since dimethyl sulfoxide (DMSO) has found utility as a diluent for various antitumor agents, at least in experimental animals, its effect on hexobarbital sleeping time was considered. Sleeping times of mice given various doses of hexobarbital were consistently prolonged when the hexobarbital was dissolved in DMSO as compared to aqueous solution. However, a further prolongation of sleeping time resulted when hexobarbital was administered in water and followed in five minutes with an injection of DMSO.

Evaluation of the excretory function of liver is routine in larger animals but more difficult in mice. However, the relatively recent introduction of indocyanine green (ICG) as a means of evaluating biliary excretion has been found adaptable. Early studies were carried out using repeated blood sampling and colorimetric analysis of dye concentration (Beckman DB-G). The repeated sampling was associated with technical difficulties reflected in variability of the results and alternatives were sought. A colorimeter with a dichromatic earpiece densitometer was made available, through the courtesy of the dye manufacturer, and the earpiece was modified to permit insertion of a mouse forelimb into the densitometer. A Beckman Linear-Log Recorder used as output was found to be superior to that provided with the densitometer. Calibration showed negligible deviation from linearity over three log cycles of concentration. In mouse studies it was found that ICG dye excretion is biphasic and apparently reflects a two-compartment physiologic system, possibly representing free dye in the plasma and plasma-protein-bound dye. The microsomal inhibitor SKF525A retards the rapid phase of excretion but apparently not the slow phase. These studies have been suspended pending receipt of our own colorimeter.

8. Feasibility Study of Data Processing Techniques

A small supplementary contract was negotiated with Mason Research Institute for the purpose of performing studies on the handling of toxicologic data obtained from investigations. The feasibility of several steps was to be considered: 1) conversion of data to machine compatible forms, 2) tabular presentation of data, 3) identification of trends and differences, 4) advanced data analysis. This work is nearing completion but is not yet available for final evaluation.

9. Microbiological Considerations in the Toxicology of Antitumor Agents

These studies, which are the primary responsibility of Dr. Howard Bruckner, are intended to provide information of the mutual interactions between antitumor agents, antimicrobial agents, and normal and pathogenic microflora.

In a study designed to evaluate by serial biopsy and microbial assay the nature of methotrexate induced gastrointestinal toxicity, three dogs were prepared by performing ileostomies. Two dogs, dosed with 2 and 5 mg/kg of MTX respectively, and biopsied, both died with signs apparently reflecting methotrexate toxicity. Lethality at these low doses might be explained by altered intestinal flora resulting from the ileostomy or from antibiotic administration.

This concept is supported by the results of antibiotic-methotrexate studies in rodents. Mortality and body weight losses resulting from combined drug administration were greater than could be accounted for by assuming independent joint action. However, this relationship holds only for oral dosing or large parenteral doses. This and bacteriological data indicate that the dose of the intestinal flora is critical for interaction and further implies that parenteral antibiotic therapy in the clinical situation may be significantly safer than oral when required simultaneously with methotrexate.

Further evidence of the indirect nature of the interaction is found in studies in which augmented toxicity occurred in mice given methotrexate beginning two days after the end of daily oral penicillin administration.

A dramatic demonstration of the indirect effects of antibiotics was achieved in a study of the effects of ampicillin on guinea pig lethality. Of guinea pigs given single intraperitoneal doses of ampicillin, 60% were dead within five days. Given fifteen equivalent doses over a five day period, all treated guinea pigs survived the course of treatment, but 60% died within five days after the end of treatment. Fecal bacteriological cultures showed an abnormally high incidence of Klebsiella, in some cases approaching a pure culture.

Significance to Bio-medical Research and the Program of the Institute:

Drug toxicity is a primary determinant of the effectiveness of cancer chemotherapy since it alone limits maximal dosage. Few if any agents tested thus far produce significant therapeutic results at doses lower than those which produce toxic signs. The fact that the toxicity of some of these drugs is schedule dependent has led to the observation that this dependence might be exploited most effectively by a consideration of causal factors.

Thus, as well as providing toxicity data on single chemotherapeutic agents, this project includes several studies intended to evaluate toxicologic relationships between drug dose spacing and cell cycle phases. These and other studies, using agents in combinations chosen on the basis of their known or suspected mechanisms of action, should improve the quantitative and qualitative predictive value of experimental models of drug toxicity and possibly suggest means of producing dramatic improvements in therapeutic benefits.

Proposed Course:

This project is intended to satisfy the continuing need for basic toxicity data and the development of new priniciples and methods. Specific experiments will be oriented towards the following objectives:

1) Descriptive toxicology of single and multiple antitumor agents.
2) Evaluation of dosage scheduling as a determinant of toxic responses to various classes of chemotherapeutic drugs.
3) Development of improved tools and methodology in toxicologic investigation.

Serial No. NCI-3509
1. Lab. of Chemical Pharmacology
2. Pharmacology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Chemical Carcinogenesis in Primates

Previous Serial Number: Same

Principal Investigator: Margaret G. Kelly

Other Investigators: Roger W. O'Gara, Richard H. Adamson, Michael D. Walker, Clyde J. Dawe, Jacqueline Whang-Peng, Vincent T. DeVita, Paul P. Carbone, John L. Lewis and William T. Reese

Cooperating Units: Laboratory of Pathology, NCI; Medicine Branch, NCI; Surgery Branch, NCI; Hazleton Laboratories, Inc., Falls Church, Virginia, Contract No. -43-65-

Man Years

Total:	2.5
Professional:	2
Others:	0.5

Project Description:

Objectives:

1. To study the effect of compounds, generally of potential carcinogenic activity, in newborn primates and to determine if the newborn primate, like the rodent, will be highly susceptible to carcinogenesis.

2. To obtain data on which to base a comparison of the response of monkeys and rodents to materials known or suspected to be carcinogenic to man, and thereby to evaluate the relative merits of rodents and primates in predicting for man.

Methods Employed:

Usual pharmacologic, biochemical, and clinical and anatomical pathologic techniques. Neurosurgical procedures, angiograms of the brain, diagnostic X-ray procedures. Cytological and immunologic techniques. In addition, breeding, nursing care of infant monkeys and special dietary regimens.

Major Findings:

1. Breeding Colony

On February 1, 1968, the breeding colony consisted of 146 monkeys including 13 Rhesus males, 61 Rhesus females, 8 Cynomolgus males, 41 Cynomolgus females, 11 African Green males, and 12 African Green females.

The breeding record and health status of the breeding colony is similar to that reported for previous years.

2. Experimental Colony (carcinogenesis)

There are currently over 200 monkeys on treatment with various carcinogens. For detailed autopsy and biopsy reports, see Annual Report of Dr. Roger O'Gara, Laboratory of Pathology, NCI (NCI-526).

As reported previously, oral and IP administration of N-nitrosodiethylamine induces liver tumors in 85-100% of the monkeys. A supply of these tumor-bearing monkeys are maintained for chemotherapy studies, drug distribution studies, and liver enzyme studies. In addition, tumor-bearing monkeys have been made available to Dr. DeVita of the Medicine Branch. A piece of liver tumor is removed at biopsy and injected SC into a dog. The dog is plasmaphoresed and the buffy coat is injected IV into the donor monkey.

In collaboration with Dr. Paul Carbone, Medicine Branch, and Dr. David Gitlin, University of Pittsburgh, blood from newborn monkeys, from monkeys with liver tumors, and from monkeys with no known liver pathology is being examined for the presence of $\underline{\alpha}$-fetoprotein. $\underline{\alpha}$-fetoprotein was present in the sera of 17 of 23 monkeys that had hepatomas on initial examination. Two of the 6 monkeys have remained persistently negative and the other 4 have become positive. Sera on 69 monkeys without tumors, treated with DENA or other carcinogens, have remained consistently negative.

No new case of leukemia has developed in the 26 monkeys on MIH (N-isopropyl-$\underline{\alpha}$-(methylhydrazino)-$\underline{p}$-toluamide. These animals have received MIH by SC injection for one to two years.

Monkeys have been started on 1-methyl-1-nitrosoureas by subcutaneous or IV route. A single SC or IC injection of newborn mice with this compound causes a high incidence of leukemia and, in rats, kidney fibrosarcomas.

The intracerebral transplantation in monkeys of the tissue culture line established by Dr. Clyde Dawe, Laboratory of Pathology, from a hepatocarcinoma infuced by nitrosodiethylamine, is continuing largely under the supervision of Dr. Michael D. Walker. Dr. Clyde Dawe gave us a subculture of the liver tumor cell line (NCLP-6) and the cells currently used for IC inoculation of monkeys are being grown in our laboratory (Dr. Bono).

Significance to Bio-medical Research and the Program of the Institute:

The induction of liver tumors in monkeys with nitrosodiethylamine provides a useful and practical primary tumor in the primate for a variety of pharmacologic studies. The successful transplantation to the brain of a tissue culture line derived from an induced hepatocarcinoma provides for the first time a system in primates for study of the effects of drugs or an intracerebral tumor, and also makes possible studies of the distribution of chemotherapeutic drugs between blood, cerebrospinal fluid, normal brain tissue, and a tumor growing in the brain.

Proposed Course:

Approximately 30 animals will be placed on each of the current carcinogenic regimens, and other carcinogens will be added as the supply permits. Combinations of chemical carcinogens with other classes of agents such as radiation and non-pathogenic viral materials will be administered in the hope of enhancing the action of the chemical carcinogen.

During the course of these studies, considerable data has accumulated which indicates that the infant monkey is 2 to 10-fold more susceptible than are young (6 months of age) or adult monkeys to the toxic effects of many of these compounds. An effort will be made during the current year to analyze the accumulated data on hand, and to study the neonatal pharmacology not only of the carcinogenic compounds under test, but also (supply permitting) of some of the new agents of current clinical interest in the management of the cancer patient.

Hepatocarcinoma lines growing in tissue culture will be maintained as a source of inoculum for implantation into other monkeys. In vitro studies of the response of this primate cell line to antitumor agents will be initiated during the coming year, either in the Laboratory of Chemical Pharmacology or some other group.

Treatment of monkeys bearing liver tumors with 6-MP, 5-FU, dichloromethotrexate and L-asparaginase will be initiated and continued with vigor.

The effect of drugs on the growth of intracerebrally-transplanted hepatocarcinoma will be studied, and neurosurgical procedures applicable to the human situation will be evaluated in the monkey. The immunologic response of the liver tumor growing in a heterologous host will be examined.

A continued effort will be made to induce leukemia with the methylhydrazine compound.

Studies of the metabolism of the various classes of carcinogens in monkeys will be continued. Such studies, it is hoped, will give more insight into susceptibility or refractoriness of primates compared to rodents.

Publications:

O'Gara, R. W., Kelly, M. G., and Kerber, W. T.: A spontaneous metastasizing carcinoma of the gall bladder in a rhesus monkey. Folic Primatologica., 6: 284-291, 1967.

Kelly, M. G., O'Gara, R. W., Walker, M. D., Dawe, C. J., Morgan, W. D., and Kerber, W. T.: Intracerebral transplantation into rhesus monkeys of cell cultures (NCLP-6) derived from a chemically-induced primate hepatocarcinoma. J. Nat. Cancer Inst., 39: 153-169, 1967.

Serial No. NCI-3516
1. Lab. of Chemical Pharmacology
2. Pharmacology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Biochemical, Pharmacologic, Toxicologic and Chemotherapeutic Aspects of Selected Antitumor Agents

Previous Serial Number: NCI-3516

Principal Investigator: J. A. R. Mead

Other Investigators: A. Goldin, J. M. Venditti, NCI. V. S. Waravdekar, I. Kline, L. D. Saslaw, L. C. Mishra and C. T. Hardesty, Microbiological Associates, Inc.

Cooperating Units: None

Man Years (computed for the 12 month period)

Total:	1 1/2
Professional:	1
Other:	1/2

Project Description:

Objectives:

Investigate the biochemical pharmacology and biochemical toxicology of antitumor agents in experimental preclinical systems to better define the parameters which influence the chemotherapeutic activity of new or established agents.

Methods Employed:

Standard laboratory procedures were employed.

Major Findings:

The studies in this area can be considered under the following headings:

A. Antifolates

B. Arabinosylcytosine

C. Sangivamycin

D. Miscellaneous

A. Antifolates

Serial No. NCI-3516

As part of a series of studies related to the pharmacologic aspects of resistance to antifolates the effects of methotrexate and dichloromethotrexate on NAD kinase activity has been studied in normal and leukemic mice. NAD kinase catalyzes the formation of NADP, the cofactor for a principal target enzyme of the 4-amino analogs of folic acid, dihydrofolate reductase. In some instances a dramatic increase has been observed in the reductase levels of tumor cells after treatment with methotrexate and dichloromethotrexate. No study has been made to see whether there is an associated increase in cofactor levels in the same tumor cells so it was appropriate to examine the capacity of the cells for the biosynthesis of NADP in the presence of antifolates. It was found that in vivo methotrexate produced marked fluctuations in splenic and hepatic NAD kinase whereas the activities were essentially unaffected by DCM. Similar results were seen in vitro and it is considered likely that the fluctuations in the kinase activity are a reflection of host toxicity rather than an effect on the dihydrofolate reductase system.

The major emphasis in the antifolate program has been in the development of additional pharmacological information about tetrahydrohomofolate. A single dose level of 1600 mg/kg was found to be lethal to 100% of a group of mice receiving the drug whereas the same dose given on four consecutive days was non-toxic to rats. Interestingly in a schedule dependency study it was found that a dose of 1600 mg/kg was not toxic to mice when given in 8 doses of 200 mg/kg every 3 hours. This would certainly indicate that the toxic effects of tetrahydrohomofolate are probably brought about by the establishment of a very high level of the drug in vivo and not by continued low levels. It was also found that in both L1210 and its antifolate-resistant variant L1210-FR8 the optimum schedule appeared to be daily treatment with an optimum dose level of between 400 to 800 mg/kg which is imposed by the toxicity of the agent to normal mice.

The activity of tetrahydrohomofolate against the Walker intramuscular tumor in rats was not remarkable. A maximum tumor weight inhibition of 50 percent was observed in one experiment. Since the maximum dose tried in the Walker system was 1600 mg/kg and this was non-toxic, a greater effect might be seen with higher doses but in the interests of conserving compound no further testing is contemplated in rats at the present.

A study has also been made of the physiological disposition and metabolism of tetrahydrohomofolate in mice. A colorimetric method has been developed which can be applied to biological materials and which measures the tetrahydrohomofolate equivalents present at various times after drug administration. The method is only valid for materials related to tetrahydrohomofolic acid which can liberate diazotizable amine at acid pH and this would eliminate oxidized products and any derivatives which might result from the transfer of metabolic C_1 units to the drug. The findings up to now indicate that the drug is readily distributed in the body after I.V. or IP administration but slowly after subcutaneous injection and very poorly after oral administration.

The physiological disposition of tetrahydrohomofolate was studied in normal BDF_1 mice after IP administration of either 400 or 800 mg/kg. Plasma levels reached a peak very rapidly and declined to 50% of the maximum level within 1-2 hours. A similar pattern was seen in other tissues but the most interesting finding was that in the kidney after a dose of 800 mg/kg there was a prolonged retention of the agent in the organ and since the toxicity of the drug has been related to an effect on kidney function the prolonged half-life of between 6 and 7 hours is suggestive of incipient kidney damage. In this connection it is appropriate to note that a dose of 800 mg/kg is not tolerated indefinitely when given daily to mice whereas the lower dose of 400 mg/kg can be administered indefinitely without noticeable ill effects. It was also significant to note that almost none of the drug appeared in the brain after IP administration at either dose level indicating quite strongly that the blood brain barrier is excluding the drug from the brain.

In a preliminary study it was found that the drug appeared to concentrate slightly in the erythrocytes and the basis for this phenomenon will be explored further. The urinary excretion of the drug was also followed and after a dose of 400 mg/kg it was found that there was rapid excretion in the urine accounting for 20% of the administered dose within 4 hours. An additional 5% of the administered dose appeared after 8 days. The fact that only 25% of the administered dose can be accounted for is strongly suggestive of extensive break down of the drug, alternatively there may be other routes of the excretion, e.g., via the bile, although no Bratton-Marshall positive material was detected in the feces. Organs such as the liver may be able to localize the agent in forms which do not react with the Bratton-Marshall reagent. In any event, it is apparent that more work will be necessary to delineate further the metabolism of tetrahydrohomofolate *in vivo*.

B. Arabinosylcytosine

Studies have been conducted on the metabolism and mechanism of action of arabinosylcytosine (ara-C). Attempts were made to reverse the megaloblastic anemia, leukopenia and thrombocytopenia which have been induced by the drug. Several compounds that have been used in the treatment of megaloblastic anemia were combined with ara-C. Among these were folic acid, citrovorum factor, Vitamin B_{12} and L-aspartic acid. Of these only L-aspartic acid had any significant effect. However, although the L-aspartic did appear to reduce the toxicity of ara-C it was not possible to gain any therapeutic advantage with the combination and no further studies along these lines are contemplated.

The phosphorylation of ara-C by pyrimidine nucleoside kinase was studied with a preparation from mouse spleen that had been purified 120-fold. The preparation was devoid of nucleoside deminase activity and phosphorylated products of ara-C were the only compounds detected in the reaction mixture. It was of some interest to note that under the conditions used, UTP appeared to be a more effective phosphate donor than ATP. However, recent work from other laboratories has shown that under optimal conditions ATP is in fact the principal phosphate donor in this system. In some other studies related to the metabolism of ara-C the possible interaction of the drug with enzymes involved in the degradation and synthesis of pyrimidine or purine nucleosides and nucleotides has been investigated. The possibility that ara-C might undergo phosphorylysis were considered but no evidence for this kind of reaction was discovered. The effect of ara-C on tissue thymidine kinase activity was not significantly changed by ara-C, although the compound increases the activity in mammalian cell culture systems.

C. Sangivamycin

A study has been started on the biochemical disposition of sangivamycin. This compound is a purine nucleoside analog which has shown some promise of being a useful antileukemic agent. Unfortunately, early in its clinical trials it was reported to have some cardiac toxicity and the clinical trials were abrogated on account of this finding. Before it was dropped, however, promising activity had been observed in acute myelogenous leukemia and for this reason it was decided to see whether it was possible to establish a biochemical basis for the reported cardiac toxicity. Sangivamycin is closely related to tubercidin and toyocomycin and these two compounds are readily phosphorylated. An investigation was conducted to determine whether sangivamycin could be phosphorylated. It has been established quite clearly that sangivamycin can be converted to a phosphorylated derivative by a kinase present in the mouse liver and also that sangivamycin will effectly inhibit the phosphorylation of adenosine present in the reaction mixture. As radiolabeled material is now available drug-induced changes will be sought specifically in the biochemical mechanisms of the heart which might result in cardiac toxicity. Tubercidin is known to be concentrated in the erythrocytes where it is phosphorylated, and this gives rise to a very prolonged half-life in blood. We shall be looking for a similar pattern with sangivamycin. We shall also study the phosphorylative uptake of the drug by human erythrocytes and hopefully human leukemic cells. These investigations have as their aim the further definition of the biochemical interactions of sangivamycin so that an attempt can be made to reduce the specific cardiac toxicity which has been attributed to the drug.

D. Miscellaneous

Two small studies were also conducted. The first involved the development of a colorimetric assay for 8-azaguanine which can be used in the presence of 8-azaxanthine. Unfortunately the method requires high concentrations of 8-azaguanine, much in excess of those likely to be achieved in vivo. This requirement severely limits the practical use of the method. The second study involved the colorimetric estimation of DNA in femoral marrow from the mouse. The basis of this method is the colorimetric determination of deoxyribonucleotides which in turn depends on the release of malonaldehyde from 2-deoxysugar moieties after periodate oxidation and subsequent condensation of the aldehyde with thiobarbituric acid. The method is approximately 40 times greater in sensitivity than the diphenylamine assay and so can be used for correspondingly smaller quantities of tissue.

Significance to Biomedical Research and the Program of the Institute

Knowledge of the physiological distribution of antitumor agents has contributed much to our increased ability to use the drugs with maximum effectiveness. This program is oriented to the needs of the Pharmacology Committee of the Acute Leukemia Task Force which sponsors investigations on the pharmacological and toxicological properties of antitumor agents.

Proposed Course of Project

The project will continue to provide relevant information on the biochemical, pharmacologic and toxicologic properties of antitumor agents and be responsive to the changing needs of the Chemotherapy Program of the Institute.

Publications:

Saslaw, L. D., Grindey, G. B., Kline, I. and Waravdekar, V. S.: "Sparing Action of Uridine on the Activity of Arabinosylcytosine with Normal and Leukemic Mice." Cancer Res. 28: 11-20, 1968.

Saslaw, L. D., Chaney, N. A., and Waravdekar, V. S.: "Colorimetric Assays for 8-Azaguanine and 8-Azaxanthine." Analytical Biochemistry 21: 147-149, 1967.

Mhatre, R. M., Saslaw, L. D., and Waravdekar, V. S.: "Effect of Methotrexate on NAD Kinase Activity in Leukaemic Mice." Nature 214: 926-927, 1967.

Grindey, G. B., Saslaw, L. D., and Waravdekar, V. S.: "Effects of Uracil Derivatives on Phosphorylation of Arabinosylcytosine." Molecular Pharmacology 4: 96-103, 1968.

Saslaw, L. D. and Waravdekar, V. S.: "The Thiobarbituric Acid Method for Deoxyribonucleosides." Methods in Enzymology 12: 108-113, 1967.

Saslaw, L. D., Chaney, N. A. and Waravdekar, V. S.: "Alterations in Cytochrome Oxidase Activity in Tissues of Normal and Leukemic Mice Treated with Arabinosylcytosine." Toxicology and Applied Pharmacology, in press.

Serial No. NCI-3517
1. Laboratory of Chemical Pharmacology
2. Pharmacology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Murine Brain Tumor Study

Previous Serial Number: None

Principal Investigators: William R. Shapiro and James I. Ausman

Other Investigators: None

Cooperating Units: Microbiologic Associates, Bethesda, Maryland

Man Years

Total:	3/4
Professional:	1/2
Others:	1/4

Project Description:

Objectives:

1. To develop a screening system for evaluation of chemotherapeutic agents which might be of use in the treatment of intracranial malignancies.
2. To evaluate intracranially and subcutaneously implanted mouse gliomas as models for testing chemotherapeutic agents.
3. To evaluate the chemotherapeutic potential of various agents against the model screening systems.
4. To develop and test optimal dosage schedules based on tumor cell kinetics and drug pharmacodynamics.
5. To develop methods of quantitation of tumor cell populations.

Methods Employed:

Usual pharmacologic and histopathologic techniques.

Major Findings:

A. There are only three stable experimental neural tumors in existence which can be transplanted successfully in experimental animals. These three tumors, ependymoblastoma of Zimmerman, glioma 26, and glioma 261-all ependymoblastomas, are being used as models for this screening system. They are maintained by subcutaneous transfer, and can be transplanted intracerebrally into large numbers of mice with reproducible median days of mortality peculiar to each tumor.

B. Various drugs have been and are being evaluated in all three tumor systems by statistical comparison of mortality times of drug treated intracerebral tumor bearing animals with non-drug treated tumor bearing controls. The responses of the different tumor systems to the drugs are being compared with each other and will be correlated with data from clinical testing to select the most predictable screening system.

1) In all three systems BCNU (1,3-bis[2-chlorethyl]-1-nitrosourea) treatment given IP as a single dose on day 2 after tumor implantation significantly prolongs the mortality time of brain tumor bearing animals. The mean increase in life span with this drug and schedule ranges from 26-49% in ependymoblastoma, 43-81% in glioma 261, and 13-32% in glioma 26 at varying dosages at or below the LD_{10}.

2) Cytoxan (cyclophosphamide) produces no consistently significant increase in mortality time in ependymoblastoma bearing animals if given IP on day 2 after tumor implantation in the brain. These results would be consistent with data of other investigators which correlated the high efficacy of BCNU against IC-L1210 leukemia with its lipid solubility in contrast to other less effective and less lipid soluble agents. A high degree of lipid solubility favors passage across the cell membranes and entry into the brain. The studies are in progress to evaluate cytoxan against gliomas 26 and 261.

3) Preliminary studies with mithramycin, the agent now being used by the Brain Tumor Task Force, have begun using the above tumor systems.

C. Doubling Time of subcutaneously transplanted ependymoblastomas (Zimmerman) based on tumor weight change were found to be 2-4 days. Experiments using radioautography are in progress to provide another approach to the doubling time value.

D. Preliminary experiments have been performed with dosage schedules based on the above doubling time using BCNU. The experiments are still in progress and the results are not yet available.

E. A method of producing single cell suspensions from solid ependymoblastoma tumors has been developed and mortality times of animals injected IC with varying log number of cells is being evaluated.

Significance to Bio-medical Research and the Program of the Institute:

The development of a screening model for testing chemotherapeutic agents against intracerebral tumors should allow the rapid evaluation of the usefulness of such compounds for the treatment of human brain tumors. By developing methods of tumor cell quantitation, a more precise analysis of effect of drugs on the tumor can be obtained. The combination of this information with the data on tumor cell kinetics and drug pharmacodynamics promises to provide clues to the optimal efficacy of individual drugs regimes to tumors.

Proposed Course:

1. Evaluation of additional chemotherapeutic agents will be made using the three intracerebrally transplanted tumor models.

2. Correlation of results with available clinical data will be made to select the most suitable system as a screen.

3. Optimization of dosage schedules based on information on tumor doubling times and drug disposition will be continued.

4. Work on quantitation of tumor cell suspensions will be carried out for use in testing situations.

Serial No. NCI 3518 (New)
1. Laboratory of Chemical Pharmacology
2. Pharmacology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies Designed to Increase the Selective Toxicity of Anticancer Drugs

Previous Serial Number: None

Principal Investigators: Philip S. Schein, David A. Cooney

Other Investigators: None

Cooperating Units: Microbiological Associates, Inc., Bethesda, Maryland

Man Years

Total:	1/2
Professional:	1/2
Others:	0

Project Description:

Objectives:

1. The biochemical and pharmacologic study of particular antitumor agents that have a specific organ-system toxicity, which lends itself to exploitation in clinical cancer chemotherapy.

2. Attempt to modify the dose limiting toxicity of specific chemotherapeutic compounds with protective agents, without concomitant loss of antitumor activity.

Major Findings:

Studies with Streptozotocin (NSC-85,998)

Previous studies have shown that the acute diabetogenic action of Streptozotocin could be prevented by the simultaneous administration of large single doses of nicotinamide. This was accomplished without any loss of antitumor activity in mice with leukemia L1210 or P-388. It was also found that toxicity could be correlated with a dose-dependent depression of nicotinamide adenine dinucleotide (NAD) concentration in the liver and in leukemia L1210 cells.

In an extended study, a single IV dose of 200 mg/kg produced a 24 hour depression of liver NAD and NADH, with a gradual return to normal concentrations by 48 hours. Injection of nicotinamide, 500 mg/kg IP, protected against NAD depression for 16 hours. Animals receiving nicotinamide alone showed significantly higher NAD values when compared to the combination treatment group.

The serum half-life of Streptozotocin was approximately 5 minutes with no drug measurable by two hours. Streptozotocin was detectable in the acid soluble fraction of liver for 20 hours, and this was unaltered by nicotinamide pretreatment.

A schedule of small serial administrations of nicotinamide over a 24 hour period was shown to be more effective than injection of the total dose just prior to Streptozotocin.

The blood sugar curve on mice after Streptozotocin treatment consists of a hyperglycemic phase by 1-3 hours, followed by a profound hypoglycemia at 7-9 hours. The animals are irreversibly diabetic by 24 hours. Measuring serum insulin levels, a dramatic rise in immuno-reactive insulin concentration is detected at the 7 hour time period, explaining the hypoglycemic phase. At 24 hours the pancreatic insulin content of Streptozotocin treated mice is very low, while those pretreated with nicotinamide showed normal concentrations. Electron microscopic examination of serial sections of the pancreatic beta cell over the 24 hour period indicated a specific attack on the endoplasmic reticulum.

The study of the diabetogenic activity of Streptozotocin has led to a series of experiments dealing with alternation in pyridine nucleotide metabolism resulting from treatment with chemotherapeutic and carcinogenic drugs. The original observation, that nicotinamide protected against both pancreatic beta cell destruction and depression of NAD concentrations induced by Streptozotocin, has developed into a unique model for selective toxicity: the prevention of toxicity without loss of antitumor activity. The active chemotherapeutic moiety of the Streptozotocin molecule is 1-methyl-1-nitrosourea, a compound which also produced significant reduction of NAD concentration in the liver and leukemia L1210 ascites cell. Nicotinamide again functioned to prevent the fall of NAD concentration in these tissues but did not reverse the antitumor activity of this compound.

Studies with 1-Methyl-1-Nitrosourea

The chemotherapeutically active moiety of the Streptozotocin molecule is 1-methyl-1-nitrosourea (MNU). Though non-diabetogenic, MNU caused a rapid reduction of NAD concentration in both mouse liver and L1210 ascites, similar to that produced by Streptozotocin. Nicotinamide pretreatment protected against this fall for about 18 hours. When normal and L1210 tumored mice were tested with MNU and the nicotinamide-MNU combination, no significant difference in either lethality or antitumor activity was noted.

The increase in survival time previously reported in nicotinamide-Streptozotocin treated mice can be explained solely in terms of reduced diabetogenic activity of the latter drug.

Nicotinamide concentrations have been determined in normal mouse liver and levels greater than control are measured for 20 hours after a single IP injection of 500 mg/kg. This corresponded to the time period in which normal NAD concentrations were maintained after the combination of nicotinamide-MNU treatment.

It has been found that MNU treatment fails to increase or decrease the activity of either the NAD glycohydrolase or NAD pyrophosphorylase enzymes.

The serum half-life of MNU was approximately 25 minutes, with no drug detectable at 75 minutes. The half-life for MNU in liver was approximately 12.5 minutes, and drug was detectable at 60 minutes, but not at two hours.

Effects of 1-Methyl-1-Nitrosourea on the Central Nervous System

MNU, 100 mg/kg IV, produced a syndrome of intermittent opisthotonos and hind limb ataxia in 50-60% of mice 2-5 hours after treatment. The mice could be repeatedly stimulated into the syndrome by loud noises or shaking. Nicotinamide pretreatment protected against this abnormality. MNU was found to cause a decrease in brain NAD, which could be prevented by pretreatment with nicotinamide. The half-life of MNU in brain is approximately 20 minutes, and drug was detectable at 60 minutes, but not at 2 hours. Brain histology has been normal on animals sacrificed days 1, 2 and 4 after treatment.

Effects of Nitrosoamines and Nitrosoureas on Liver NAD Concentrations

N-nitrosodimethylamine, N-nitrosodiethylamine, and 2-chloro-N-methyl-N-nitrosoethylamine caused a marked dose-related reduction in the NAD content of the liver, but not of L1210 ascites cells, BCNU, CCNU, CFNU, and Norbornyl CCNU did not alter NAD concentrations, and neither their toxicity nor antitumor activity was changed by pretreatment with nicotinamide.

The Temporal Effects of Cytosine Arabinoside Treatment on the Synthesis of DNA in Mouse Bone Marrow Cells and Duodenal Mucosa

Both hematopoietic tissue and duodenal mucosa demonstrated a profound reductio in T^3-TdR uptake for periods of 6-8 hours, with a gradual return to normal uptake by 12-16 hours. Attempts to demonstrate toxicity priming has been unsuccessful to date.

Significance to Bio-medical Research and the Program of the Institute:

The observation that Nicotinamide can protect against both the diabetes and NAD depression induced by NSC-85998, Streptozotocin treatment while increasing the life span of NSC-85998 treated tumor bearing mice, represents a unique model of selective toxicity: the modification of toxicity without loss of activity. The fact that 1-methyl-1-nitrosourea is not in itself diabetogenic would strongly suggest that the glucose on the Streptozotocin molecule is acting as a carrier for selective transport of the cytotoxic moiety into the pancreatic beta cell. This observation might lead to the development of a series of compounds that could be effective against functioning islet cell tumors in man.

By the examination of specific enzymes and intermediates in the synthesis and degradation of NAD it is hoped that the exact aberration in NAD metabolism will be located. This finding will lend itself to estimation of the relative importance of various pathways in pyridine nucleotide biochemistry, against which specific attack may be directed.

Proposed Course:

1. In an attempt to develop a series of compounds which will have specific activity against functioning pancreatic β-cell tumors, structural analogues of Streptozotocin are being synthesized for evaluation of diabetogenic potency.

2. The possible relationship of the separate phenomenon of carcinogenesis of nitrosoamines and nitrosoureas, and their ability to depress NAD concentrations, is currently undergoing evaluation.

Publications

1. Schein, P. S., Cooney, D. A. and Vernon, M. L. The Use of Nicotinamide to Modify the Toxicity of Streptozotocin Diabetes without Loss of Antitumor Activity. Cancer Res. 27: 2324-2332, 1967.

2. Schein, P. S., and Loftus, S. Streptozotocin: Depression of Mouse Liver Pyridine Nucleotides. Cancer Res. In Press.

3. Rakieten, N., Gordon, B. S., Cooney, D. A., Davis, R. D. and Schein, P. S. The Renal Tumorigenic Action of Streptozotocin in Rats. Cancer Chemotherapy Rept. In Press.

Serial No. NCI-3519 (New)
1. Lab. of Chemical Pharmacology
2. Pharmacology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Preclinical Toxicology of Potential Cancer Chemotherapeutic Agents

Previous Serial Number: None

Principal Investigators: Philip S. Schein, David A. Cooney, and Ruth D. Davis

Other Investigators: None

Cooperating Units: Mason Research Institute, Worcester, Massachusetts; South Shore Analytical & Research Laboratory, Inc., Islip, New York; Charles Pfizer & Co., Inc., Maywood, New Jersey; Arthur D. Little, Inc., Cambridge, Massachusetts; Stanford Research Institute, Menlo Park, California; Illinois Institute of Technology Research Institute, Chicago, Illinois

Man Years

Total:	2-1/2
Professional:	1-1/2
Others:	1

Project Description:

Objectives:

It has been the purpose of these toxicology studies to characterize the qualitative and quantitative toxicity of potential clinical cancer chemotherapeutic compounds and to delineate a safe initial clinical dose. Drugs which are promoted to the IVA category by the Decision Point Network Staff of the Chemotherapy Program, NCI, were thoroughly evaluated according to a protocol entitled "An Outline of Procedures for Preliminary Toxicologic and Pharmacologic Evaluation of Experimental Cancer Chemotherapeutic Agents".

A new toxicologic protocol has been developed to facilitate the more rapid generation of preclinical data. It is divided into three basic types as outlined below.

Type I Toxicology

1. Purpose:

   a) To define the qualitative and quantitative toxicity of potential clinical cancer chemotherapeutic compounds. The toxicity will be characterized as to predictability, reversibility, relationship to cell cycle kinetics, and temporal relationships to the end of dosing (acute or delayed onset).

   b) To obtain in the dog, a maximum tolerated dose for a single injection, 24 hour infusion, and five day daily treatments, each regimen repeated at least one time.

2. If the toxicity proves predictable by the usual observations, clinical biochemical tests or hematological observations, and proves to be reversible, the compound will be considered for a cautious clinical trial in one of a very few institutions. Simultaneous with such a clinical trial, Type II Toxicology will proceed. Alternatively, if unpredictable irreversible toxicity is elicited, it is likely that the compound will be dropped as a candidate for clinical trial.

Type II Toxicology

1. Purpose:

   To elucidate the toxicity in a second large animal species (generally a primate) and to explore other dose schedules and regimens that appear applicable on the basis of experimental animal tumor studies and on the basis of data forthcoming from Phase I clinical studies.

Type III Toxicology

In view of projected long term use of a given compound in the clinic for extended maintenance therapy and in the treatment of non-neoplastic diseases, chronic toxicity will be studied in a suitable species for periods up to one year. In addition, protocols will be designed to study specific compounds in combination with other drugs to investigate the potential synergistic toxic action on specific organ systems or to search for independent toxicities.

Major Findings:

At the present time, a contract program at the Stanford Research Institute has been studying the chronic toxicity of long term administration of Methotrexate, Vinblastine, 5-fluorouracil and phenylalanine mustard in beagle dogs and rhesus monkeys. The animals have been on test for eight months with minimally toxic doses. The chief toxicity to date for all drugs has been bone marrow depression which has demonstrated good reversibility despite the long course of serial insult. Periodic bone marrow biopsies are being performed to look for evidence of myelofibrosis or aplasia. The MTX treated monkeys have shown varying degrees of hepatocellular destruction and it is anticipated that the animals will demonstrate hepatic fibrosis by time of autopsy. It has been shown that the FIGLU (formiminoglutamic acid) determination on urine is the most sensitive indicator of developing toxicity with abnormalities appearing before changes in white count of serum transaminase levels.

At the Illinois Institute of Technology Research Institute, studies are underway to determine the efficacy of the beagle dog as a predictor of schedule dependent toxicity using the fluorinated pyrimidines 5-fluorouracil and 2'-deoxy-5-fluorouridine. It has been reported previously in clinical studies that the toxicity of 5-FU was significantly decreased when administered by continuous intravenous infusion, while that of 5-FUDR was increased approximately twenty-fold in an inverse relationship between length of infusion and tolerated dose. Using the beagle dog, the toxicity of 5-FU and 5-FUDR was decreased when administered for 12-36 hours in an intravenous infusion thus failing to correlate with the clinical situation. This can be explained in part by the differences in qualitative toxicities produced by these drugs in the two species. Whereas in man, the bone marrow and gastrointestinal mucosa are the most sensitive organ systems, the liver and central nervous system of the dog show primary susceptibility with very little leucopenia appearing with a wide range of doses and schedules of administration.

Of those compounds tested during the last year, several have been of interest from the standpoint of toxicology.

NSC-13002, Acridine, 9-[p-(dimethylamino)anilino]-, has proved to be a potent central nervous system and pulmonary toxin. Single doses of 5-15 mg/kg in both the dog and monkey produced severe convulsions and death while low-dose daily treatment produced a syndrome of tremors and ataxia. Pathology on the brain of monkeys revealed neuronal degeneration. Pulmonary complications consisted of massive emboli, acute hemorrhages and interstitial pneumonitis. Two dogs demonstrated pulmonary neoplastic changes including a focus of anaplastic bronchogenic carcinoma. Additional toxicities included foci of heptocellular necrosis and fatty metamorphosis, anemia, leucopenia and thrombophlebitis. In view of the severe central nervous system toxicity and the induction of pulmonary emboli and neoplasm, this compound was not recommended for clinical trial.

NSC-79423, Ethanol, 2,2'-[(p-nitroso-phenyl)imino]di-, dimethanesulfonate has been shown to cause a syndrome of congestive heart failure in both the dog and monkey. The course of this syndrome is characterized by the development of hypotension, râles, pleural effusion, EKG abnormalities and death by cardiac arrest. The dose level required to produce this syndrome is very close to what is considered to be tolerated in these species.

Additional studies have shown the syndrome to be partially corrected by use of intravenous Digoxin. It has been recommended that the compound not be used in clinical trials. Compounds of similar chemical structure will be screened for similar cardiac toxicity prior to further toxicologic evaluation.

NSC-109,229, L-Asparaginase, a compound of significant clinical interest in view of its reported clinical antileukemic activity without toxicity, has been studied extensively. Antigenicity studies have demonstrated the ability to induce acute anaphylaxis in sensitized guinea pigs, but this phenomenon could not be produced in the rhesus monkey. Evidence of production of precipitating antibodies has been found in rabbits. In rhesus monkeys, five of the eight batches of E. coli derived enzyme have produced temperature elevations 1-5 hours after dosing. Serial peripheral white counts have demonstrated a consistent rise at 2 hours, which at times is preceded by a transient fall. This biphasic pattern can be seen with bacterial endotoxin. The most consistent toxicity found in all batches has been anemia without evidence of a hemolytic phenomenon. Significant leucopenia was produced only with batches of exceptionally low specific activity.

Many of the monkeys demonstrated dramatic fatty vacuolization of hepatocytes which was independent of dose or specific activity, and was indicated by an increase in BSP retention. Varying degrees of colitis were produced using several batches, and caused death of one monkey. Beagle dogs appeared to be quite resistant to any toxic effect of the enzyme other than mild anemia.

NSC-1390, Allopurinol - Mason Research Institute
A sodium salt of allopurinol was formulated and tested by the intravenous route of administration in rhesus monkeys and beagle dogs. Anemia, leucocytosis, BSP retention and elevation of BUN were noted at toxic dose levels. In the monkey, histopathology revealed an interstitial nephritis, and crystals which were subsequently identified as Alloxanthine were found in the renal collecting tubules of animals at the two highest dosages. Elevations of PBI and BEI without clinical signs of hyperthyroidism was demonstrated in both species. Thyroid histology was unremarkable.

NSC-13128, Nicotinamide - South Shore Analytical & Research Laboratory, Inc.
This toxicologic evaluation in beagle dogs was necessitated by the use of large doses of this compound in conjunction with the initial clinical trials of Streptozotocin (NSC-85998). The most dramatic toxicity involved the eyes where conjunctivitis and corneal discoloration was observed. Mild reactive hepatitis was accompanied by BSP retention and elevations of serum transaminase. Mild transient hyperglycemia was noted without obvious pancreatic beta cell abnormalities. The bone marrow reacted with a development of leucocytosis.

NSC-24559, Mithramycin - Charles Pfizer & Company
The intravenous toxicity of the new standard lot of Mithramycin (3580-53) as tested in mouse and dog was comparable to the previous standard material. Concurrent administration of Vitamin K with toxic doses of Mithramycin failed

to prevent the generalized hemorrhagic phenomenon which appeared directly attributable to thrombocytopenia. Administration of the drug on an every other day schedule failed to influence the toxicity of a given total dose, as compared to daily administration.

NSC-56054, Pseudourea, 2,2'-(9,10-anthrylenedimethylene)bis-[2-thio-, dihydrochloride - Mason Research Institute
Pseudothiourea and its parental vehicle, 25% propylene glycol in 6.67% dextrose, were evaluated in beagle dogs using a weekly schedule. Vomiting, and mild anemia which reversed on continued therapy, were the only significant features of this study. The vehicle was found to be non-toxic.

NSC-61487, Carbonic acid, bis(p-aminophenyl) ester, dihydrochloride - South Shore Analytical & Research Laboratory, Inc.
Anemia and leucopenia were present in both dogs and monkeys. Dramatic methemoglobin production, leading to cyanosis and dyspnea, was documented in several dogs. It was demonstrated that the compound was metabolized at least in part to p-aminophenol, and the administration of methylene blue partially corrected the red cell oxidative changes. The gastrointestinal tract showed petechial hemorrhages; liver damage was evidenced by elevation of serum transaminase levels. Hematuria was a recurring sign at toxic dose levels.

NSC-70845, Nogalamycin - Mason Research Institute
In previous studies employing daily injections, this compound produced thickening of the walls of small and medium sized blood vessels of beagle dogs. This was accompanied by pulmonary edema and fibrous deposition in the alveoli. In an attempt to circumvent these changes, a one hour infusion was administered which resulted in intravascular coagulation and massive distention of pulmonary veins. A supplementary study was performed to determine the effect of a 12 hour infusion. One of two dogs showed inflammation and thrombus formation in the arterioles of the lung, with inflammation of the surrounding lung tissue. In view of this compound's predilection for pulmonary thrombosis, it was recommended that it not be used in clinical trials.

NSC-79037, Urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso (CCNU) - Mason Research Institute
CCNU has been studied in rhesus monkeys and beagle dogs by the oral route, employing daily and weekly schedules. Delayed liver damage was the principal dose limiting toxicity and an inverse relationship between size of dose and length of time that transpired before first elevation of SGPT was demonstrated. Histopathology showed a spectrum that encompassed cloudy swelling, hepatocellular necrosis, fatty changes and cirrhosis. In addition, there was significant bone marrow hypoplasia with leucopenia, anemia and delayed thrombocytopenia. Lymphoid tissue was atrophic. Renal toxicity was evident in both species but more marked in the monkey.

NSC-82196, Imidazole-4(or 5)-carboxamide)5(or 4)-3[3,3-bis(2-chloroethyl) -1 triazeno]- South Shore Analytical & Research Laboratory, Inc.
NSC-82196, which has shown excellent oral activity against L1210 ascitic leukemia, underwent toxicologic evaluation by both a daily and a weekly schedule. Bone marrow toxicity was manifested by anemia and leucopenia which at moderately high dose levels demonstrated good reversibility. Delayed liver damage was the chief dose limiting toxicity and was manifested by increased serum transaminase and alkaline phosphatase levels, which at high doses went on to complete liver failure and frank ascites.

NSC-88106, Urea, 1-(2-chloroethyl)-3-(2-norbornyl)-1-nitroso-
Mason Research Institute
NSC-88106 has undergone toxicologic evaluation in beagle dogs and rhesus monkeys by the oral route using both daily and weekly schedules. Marked anemia and leucopenia were observed in the dogs, while monkeys demonstrated only delayed anemia. Atrophic and hemorrhagic lymph nodes were observed in both species. Similar to the cyclohexyl derivative, NSC-79037, the principal dose limiting toxicity was delayed liver damage; this was evidenced by marked elevations in BSP retention, serum transaminase and alkaline phosphatase values. Histology demonstrated cellular necrosis, fatty changes and cirrhosis.

The monkey's kidney showed greater susceptibility than that of the dog, and showed degenerated tubules which correlated with delayed elevations of BUN.

The qualitative and quantitative toxicities of this compound cannot be readily distinguished from that of its congeners BCNU and CCNU.

NSC-92188, Isoquinaldaldehyde, thiosemicarbazone - Mason Research Institute
This thiosemicarbazone has undergone evaluation by the oral route. The principal dose limiting toxicity in both the dog and monkey involved the liver. BSP retention was the most sensitive indicator and fatty infiltration was the histologic correlate. Leucopenia was present in both species; anemia, which showed the usual characteristics of a hemolytic process, was noted in the monkey. Diarrhea, emesis, and oral ulceration were the chief features of gastrointestinal toxicity.

NSC-94100, D-Mannitol, 1,6-dibromo-1,6-dideoxy-
South Shore Analytical & Research Laboratory, Inc.
This halogenated polyalcohol, effective in the treatment of chronic myelogenous leukemia, has undergone long term evaluation by the oral route. Dose related myelosuppression was noted in all animals with primary involvement of the white cell series. Mucosal ulcerations and hemorrhages of the intestinal tract, and chemical evidence of liver toxicity were noted in both monkeys and dogs at toxic dose levels. Azotemia and proteinuria were noted only in the monkeys.

NSC-100,844, Saramycetin - South Shore Analytical & Research Laboratory, Inc. A supplementary study was conducted to test the effect of subcutaneous administration, the proposed clinical route. There was no gross or histologic evidence of local irritation at the injection site. No new toxicologic features were demonstrated using the subcutaneous route, when compared with the previous studies using intravenous administration.

NSC-104,800, Dibromodulcitol - South Shore Analytical & Research Laboratory, Inc. This halogenated polyalcohol has undergone long term toxicity testing. Dose-related myelosuppression to the extent of agranulocytosis was noted in both dogs and monkeys. Abnormalities in hepatic function occurred at toxic dose levels and fatty vacuolization of hepatocytes was noted in affected monkeys. Diarrhea associated with mucosal and submucosal damage of the gastrointestinal tract was observed in dogs receiving the highest dose levels. An area of carcinoma *in situ* was noted in the uterus of one dog and was thought to be an incidental finding, unrelated to drug treatment.

Protocol Studies in Progress

NSC-1895	Guanazole	South Shore Analytical & Res. Lab.
NSC-17251E	11β,17,21-Trihydroxy pregn-4-3ne-3,20-dione 21-(2-chloro-4-nitro-benzoate	Arthur D. Little, Inc.
NSC-17256E	Pregn-4-ene-3,11-20-trione, 6α-methyl-	Mason Research Institute
NSC-18429	Aniline, *N*,*N*-bis (2-chloroethyl)-	Mason Research Institute
NSC-71261	9H-Purine-6-thiol, 2-amino-9-(2-deoxy-β-D-ribofuranosyl)-, hydrate	Charles Pfizer & Company
NSC-77471	Mitochromin	Charles Pfizer & Company
NSC-83265	L-Alanine, 3-(tritylthio)-	Arthur D. Little, Inc.
NSC-87974	Urea, 3-cyclohexyl -1-(2-fluoroethyl)-1-nitroso-	Mason Research Institute
NSC-104,469E	Cholest-5-en, 3β-hydroxy-, [*p*-[bis (2-chloroethyl)amino] phenyl]-, acetate	Mason Research Institute

NSC-403,169	Acronycine	Mason Research Institute

Special Studies in Progress

NSC-82196	Imidazole4(or 5)-carboxamide(5(or 4)-3 [3,3-bis(2-chloroethyl) -1 triazeno]-	Arthur D. Little, Inc.
	Influence of schedule on toxicity in beagles.	
NSC-82151	Daunomycin	Charles Pfizer & Company
	Influence of schedule on toxicity in beagles.	
NSC-63878	Cytosine, 1-β-D-arabinofuranosyl-, hydrochloride	South Shore Analytical & Res. Lab., Inc.
	Influence of topical application on toxicity in mice and rats.	
NSC-85998	Streptozotocin	South Shore Analytical & Res. Lab., Inc.
	Effect of alterations of schedules in method of administratio on the diabetogenicity in beagles. Requirement of the citrate buffer pH four in the practical use of NSC-85998. Influence of the prevention of diabetes, by pretreatment with nicotinamide, on the subsequent development of renal adenomas in rats.	
NSC-109,229	L-Asparaginase	South Shore Analytical & Res. Lab., Inc.
	Effect of chronic (90 day) daily treatment in beagle dogs.	
Propylene glycol		Mason Research Institute
	Effects of varying concentrations of propylene glycol in saline on osmotic fragility of normal human red blood cells.	
NSC-56410	Porfiromycin	South Shore Analytical & Res. Lab., Inc.

	Influence of long term weekly treatment on toxicity in beagle dogs.	
NSC-32946	Methyl-GAG	Arthur D. Little, Inc.
	Comparison in the beagle dog of 24 hour infusion and quick intravenous push on the toxicity of the single maximum tolerated dose.	
NSC-104,967	Acetanilide, 3'-[bis (2-hydroxyethyl)amino]-, di-, dimethanesulfonate (ester)	Arthur D. Little, Inc.
	To ascertain the potential cardiac toxicity in beagle dogs of this NAMS (NSC-79423) analog.	
NSC-79037	Ethanol, 2,2'-[(*p*-nitrosophenyl)imino]di-, dimethanesulfonate	IIT Research Institute
	The pathogenesis of the delayed liver toxicity is being studied with an augmented battery of liver function tests and repeated percutaneous liver biopsies. Special light and electron microscopy, with special stains, of biopsy specimens are being performed.	

Significance to Bio-medical Research and the Program of the Institute:

1. The clinical use of drugs developed under the chemotherapy program is dependent on the pharmacologic data developed in preclinical species.

2. Current regulations require that toxicologic data developed in preclinical species be filed with the Food and Drug Administration prior to clinical trials.

3. Unforseen or problematic toxicity encountered in the clinic is referred back to preclinical species in an effort to elucidate its mechanism and possibly to counteract it.

Proposed Course:

This project will continue to characterize qualitative and quantitative toxicity of potential cancer chemotherapeutic agents and to suggest an initial clinical dose.

Serial No. NCI-3502
1. Lab. of Chemical Pharmacology
2. Biochemical Pharmacology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on Folic Acid and Antagonists

Previous Serial Number: Same

Principal Investigators: Israel D. Goldman, Norman S. Lichtenstein, and Vincent T. Oliverio

Cooperating Units: None

Man Years

Total:	3.0
Professional:	2.5
Others:	.5

Objectives:

To study the kinetics of entry into and passage across biological membranes of methotrexate (MTX) and folic acid in L1210 leukemia tissue culture cells.

Methods Employed:

For transport studies, L1210 leukemia cells were grown at 37° C in a modified spinner medium supplemented with fetal calf serum. Cells were collected by centrifugation, suspended in buffered solution, and incubated in flasks under a stream of 95% O_2-%5 CO_2 with mechanical stirring. Following addition of appropriate test substances, samples of the suspension were removed at certain time intervals, centrifuged and the cell plug weighed on an electronbalance. Radioactivity was determined in samples by liquid scintillation counting.

Major Findings:

Studies over the past year have led to the characterization of a carrier mediated transport system for MTX in the L1210 leukemia cell capable of transporting MTX against an electrochemical potential gradient. This transport mechanism is shared, at least in part, by the folates normally present under physiological conditions, such as folic and folinic acids. Several unique properties of MTX transport in this cell system indicate that MTX be a useful model substrate for the study of folate transport in general.

Riboflavon appears to enhance influx of MTX and preliminary studies suggest that this may be related to alteration of the MTX-carrier mobility. Also, metabolic inhibitors enhance uptake in these cells by at least two mechanisms. First, unidirectional influx is accelerated and second, efflux is inhibited. These observations are wholly dependent upon the absence of energy substrate. In their presence, uptake is retarded.

The transport of folic acid into the L1210 leukemia cells is carrier mediated since it satisfies the parameters of a) saturation, b) structural specificity, c) temperature sensitivity, and d) counter transport. Saturation is demonstrated with a K_m of about 100 μM and a V max 1 mμM/min/gm dry weight. Specificity is shown by the competitive inhibition between MTX and folic acid. There is marked temperature sensitivity with a Q_{10} of 5.4. Preloading cells with folic acid and then adding MTX, folinic acid or excess non-radioactive folic acid which causes counter transport of folic acid. Incubation of the cells in an atmosphere of N_2 causes a two to five-fold stimulation of uptake of folic acid. The addition of glucose to the median prevents this stimulated uptake of folic acid in N_2 atmosphere. It appears that approximately 25% of the folic acid is metabolized in these studies as indicated by column chromatography on DEAE cellulose.

Significance to Bio-medical Research and the Program of the Institute:

Methotrexate is one of the most important therapeutic agents in the treatment of various malignancies. Its use has resulted in cures of choriocarcinoma and in prolonged remission maintenance of actue lymphocytic leukemia.

Since transport of MTX into human white cells is slow, it is hoped that these studies may suggest alterations in MTX structure or adjuncts in the method of administration which will lead to enhanced uptake and impaired chemotherapeutic efficacy.

Proposed Course:

1. Continue studies of the transport kinetics of folic acid and MTX into L1210 leukemia cells and resistant variants.

2. Extend studies of MTX and folic acid transport in L1210 leukemia cells into white blood cells from a variety of patients *in vitro*.

Serial No. NCI-3512
1. Lab. of Chem. Pharmacology
2. Biochemical Pharmacology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Biochemical Pharmacology of Newer Antitumor Agents

Previous Serial Number: Same

Principal Investigators: Richard H. Adamson, Larry G. Hart, and Vincent T. Oliverio

Other Investigators: None

Cooperating Units: None

Man Years

Total:	4.0
Professional:	2.5
Others:	1.5

Project Description:

Objectives:

To study the biochemistry and pharmacology of 3-cyclohexyl-1-(2-chloroethyl)-1-nitrosourea (CCNU), cyclophosphamide (cytoxan), anthramycin methyl ether (AME), camptothecin, L-asparaginase, L-asparagine, and related congeners of the latter compounds. Also, to examine the biliary excretion of certain organic compounds in newborn and adult dogs and observe the effects of phenobarbital pretreatment on the biliary excretion of certain acidic drugs in rats.

Methods Employed:

Analysis of blood, bile, cerebrospinal fluid, expired carbon dioxide, and fecal material of animals following treatment with 3-cyclohexyl-1-(2-chloroethyl)-1-nitrosourea (CCNU). Isolation of the drug and its metabolites by established and/or new developed extraction and ion-exchange column techniques. Identification and confirmation of compounds by elemental, UV, and IR analysis, thin layer and paper chromatography, high voltage electrophoresis, and comparative column chromatography. The modified Schöniger oxygen flask combustion techniques in combination with liquid scintillation counting and autoradiography was employed for radioactive assays of radioactive CCNU. With cytoxan, experimental mice were pre-injected with either SKF-525A (β-diethylaminoethyl diphenylpropylacetic acid) or phenobarbital and statistical analysis made of the results of host lethality. In anthramycin (AME) experiments, CDF_1 male mice were injected i.p. with 0.15 mg/kg AME at either 30 minutes, 1 hour, 4 hours, or daily for 4 days prior to i.p. hexobarbital, 150 mg/kg. Blood pressure and respiration changes were recorded with a polygraph in anesthetized dogs which had received doses up to 1 mg/kg of AME.

Various organic solvents were employed to extract camptothecin from physiological fluids, and ultraviolet and fluorescence spectroscopy were employed to determine quantitatively the amount of camptothecin and its biotransformation products in these fluids.

L1210 and L5178Y cells grown in RPMI-1630 medium supplemented with fetal calf serum were harvested and counted with a Coulter counter. L-asparagine and several structurally related compounds were tested for cytotoxic effects against the growth of 1×10^5 cells for three days.

In the biliary excretion studies, adult male rats (pretreated three days with either phenobarbital 40 mg/kg b.i.d. or saline) were functionally nephrectomized and bile ducts cannulated. In adult and newborn mongrel dogs with cannulated bile ducts, the concentrations of administered indocyanine green were meausred colorimetrically in bile and plasma samples.

Major Findings:

During the past year an intensive effort was made to further identify the metabolic products of 3-cyclohexyl-1-(2-chloroethyl)-1-nitrosourea (CCNU) in rodents, dogs, and monkeys using CCNU with ^{14}C-label in three separate positions of the molecule, the ethylene, carbonyl, and cyclohexyl moieties.

Following a single parenteral injection of 50 mg/kg ^{14}C-cyclohexyl labeled CCNU in mice, thin-layer chromatography in several solvent systems of the 1 and 2-hour urine samples yielded 6 radioactive spots in addition to a trace of parent CCNU. Two radioactive spots were identified as dicyclohexylurea (3-5%) and cyclohexylamine (17-20%). The remaining unidentified radioactive spots appeared to be more polar in migrating characteristics. Control mouse urine with added radioactive CCNU which was incubated at 37° C for 30 minutes did not yield the radioactive metabolites isolated from in vivo experiments. The dog similarly yielded dicyclohexylurea and cyclohexylamine in the urine following I.V. CCNU.

We further examined the plasma and concurrent CSF levels of intact CCNU and radioactivity in dogs given a single I.V. dose of cyclohexyl-^{14}C-label CCNU or ethylene-^{14}C label CCNU. With the cyclohexyl labeled CCNU, radioactivity entered the CSF rapidly, but was only about 55% of the plasma level between 30 and 60 minutes after injection of the drug. Most of this radioactivity represented ether non-extractable products of the parent compound since the rate of decline of the ether extractable intact CCNU in both plasma and CSF was very rapid with a half-life of about 15-20 minutes.

From these studies in dogs it is evident that the ethylene half of the CCNU molecule has no difficulty crossing the blood-brain barrier while the cyclohexyl moiety, by virtue of extensive plasma protein binding (45%-60%), is concentrated in the CSF to a much lesser extent. In dogs given the ethylene-^{14}C labeled CCNU, no plasma protein binding was observed. It appears, therefore, since the parent compound has an extremely short half-life, that only a minute portion of the radioactivity in the CSF represents entry of the intact parent compound.

We envision the in vivo breakdown of CCNU as following the proposed in vitro chemical breakdown of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) by Johnston and co-workers at Sourthern Research Institute and Loo at the M. D. Anderson Hospital and Tumor Institute. During this breakdown, the formation of an intermediate isocyanate is postulated. We have tested cyclohexyl isocyanate, cyclohexylamine, and dicyclohexylurea against L1210 mouse leukemia and have found these metabolites of CCNU to be devoid of antitumor activity.

In studies on the effect of pretreatment of rodents with the microsomal inhibitor SKF-525A (β-diethylaminoethyl diphenylpropylacetic acid) on lethality following treatment with cytoxan, it appears that the parent cytoxan molecule may be more toxic to the host than its activation product(s). In mice it was observed that a greater mortality and a marked earlier onset of death resulted in mice pretreated with SKF-525A. Further, there is a suggestion that the host toxicity may be different in kind from the cytotoxicity produced by the therapeutically effective activation products. In contrast to the studies with SKF-525A, there were not appreciable differences between phenobarbital and saline pretreated mice with respect to either onset of mortality or total mortality. Since phenobarbital increases the activity of microsomal enzymes, one might expect a decrease in host mortality by increasing the rate of metabolism of the parent cytoxan molecule.

The effect of phenobarbital pretreatment on the biliary excretion in rats of chlorothrazide, indocyanine green (ICG) and phenol red was studied. Pretreatment with phenobarbital resulted in enhanced biliary excretion of ICG but not of chlorothiazide phenol red and its glucuronide. Certain differences between male and female rats were noted in the biliary flow rate and quantity of the acids excreted. It was proposed that the mechanism by which phenobarbital increased bile flow and excretion was to increase the synthesis and excretion of bile acids from the precursor cholesterol. The biliary excretion of ICG was also studied in dogs to determine whether the rate and amount excreted was significantly lower in the newborn dog than the adult animal. In newborn dogs of 10-18 days in age, ICG appeared to be excreted at a rate similar to that of adult dogs and no sex differences were apparent in either groups of animals.

Preliminary studies were undertaken to evaluate the antitumor activity of the plant alkaloid camptothecin against various rodent tumor systems and to develop spectrophotometric and fluorometric assays for estimation of the drug in biological tissues and fluids.

It was previously reported by others that camptothecin showed marked activity in L1210 leukemia in mice and the Walker 256 (IM) tumor in rats. In our studies, antitumor activity in terms of increasing median survival time of L1210 leukemia-bearing mice was greater when the drug was given every four days rather than daily. The optimal dose was 50 mg/kg and resulted in an increase in median survival over control of 153%. The drug was extracted from acidified plasma, urine, and bile with ethyl acetate in 95%-100% recoveries. Using ultraviolet spectroscopy and measuring the optical density at 358-363 mμ, 1-2 ug/ml of drug could be quantitatively detected.

Work continued on the antitumor antibiotic anthramycin methyl ether (AME). Like actinomycin D, it was active against mouse leukemia P388, but inactive against P388/38280. AME had activity against the following experimental tumors: leukemia L1210, leukemia K1964, leukemia L5178Y, leukemia P388/UCR and plasma cell LCP. AME had little or no activity in two reticulum cell sarcomas, the plasma cell YPC-1 or the mast cell tumor P815.

Effects of AME were studied on other pharmacologic parameters. Hexobarbital sleeping times were doubled in mice receiving daily injections of AME for four days while single injections at times varying from 30 minutes to four hours prior to hexobarbital had no effect on sleeping time. AME did not affect the blood pressure, respiration, or EKG in anesthetized dogs, nor did it alter the blood pressure response to various autonomic stimulants.

AME markedly inhibited uptake of tritated uridine in both L1210 and P388 leukemic cells in vivo. AME also interfered with uptake of tritiated thymidine by L1210 cells. Spectral studies indicated that the ultraviolet absorptions maximum of AME was shifted to longer wave lengths following interactions with DNA, but deoxyguanosine had no effect on the absorption maximum of AME. The anthranilic acid moiety did not form complexes with DNA or deoxyguanosine nor did it have cytotoxic effects in tissue culture in doses up to 1,000 times that of the ID_{50} for AME.

During the past year studies were initiated on the pharmacology and antitumor activity of L-asparaginase and asparagine analogs. L-asparaginase was active against mouse leukemia L5178Y, but L-asparagine (200-400 mg/kg) did not affect the growth of this tumor as measured by median survival time. Also, treatment of mice bearing L5178Y with L-asparagine for several transplant generations did not make the tumor dependent on this source, nor was the tumor more sensitive to L-asparaginase.

Several compounds structurally related to L-asparagine were tested for antitumor activity in vivo and in vitro against leukemia L5178Y. L-asparagine, D-asparagine, diaminobutyric acid, glycylglycine, and S-methyl-L-cysteine were inactive. However, S-carbamyl-L-cysteine (SCC) showed minimal activity in increasing survival time (30%) but also was active in L1210 (40%). The ID_{50} for SCC in tissue culture against L5178Y and L1210 was about 10 μg/ml.

L-asparaginase was also evaluated for possible teratogenic activity in the rabbit. Results thus far suggest that L-asparaginase causes resorptions and malformations of the fetus.

Significance to Bio-medical Research and the Program of the Institute:

Studies of the distribution rate of disappearance and metabolism of these agents may provide some clues as to the mechanism of action associated with the antitumor process. The biliary excretion studies were designed to better our understanding of hepatic excretory function in newborn and adult animals and its relationship to therapeutic effectiveness of drugs. The results of the studies may serve as a guide to the design and synthesis of more active and less toxic analogs.

Proposed Course:

1. Continue studies of pharmacologic disposition of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea in man and animals.

2. Study the pharmacologic disposition of camptothecin in animals.

3. Further study the effects of microsomal inhibitors and stimulators on host toxicity in animals that more closely resemble man in the ability to metabolize cytoxan and studies of these agents on the cytotoxic effects of cytoxan on animal tumor systems.

4. Further investigate biliary secretion of organic anions in neonatal period and on secretion of compounds that require prior hepatic conjugation.

5. To further study effects of chronic drug treatment on the parameters of hepatic metabolism and bile excretion of drugs.

6. Continue studies on the pharmacologic dispositions of L-asparaginase and its possible teratogenic effects in several animal species.

7. Continue comparative studies of drug disposition especially with regard to metabolism, biliary excretion, and ability to cross the blood-brain barrier.

8. To begin studies of the distribution of radioactive labeled drugs in rodents using the whole body autoradiographic technique at sub-zero temperatures.

Publications:

1. Adamson, R. H.: Drug metabolism in murine vertebrates. Fed. Proc. 26: 1047-1055, 1967.
2. Adamson, R. H.: Antitumor activity of S-carbamyl-L-cysteine. Nature 47: 751-752, 1968.
3. Adamson, R. H., Hart, L. G., DeVita, V. T., and Oliverio, V. T.: Antitumor activity and some pharmacologic properties of anthramycin methyl ether. Cancer Res. 28: 343-347, 1968.
4. Davidson, J. D. and Oliverio, V. T.: Tritium and carbon-^{14} by oxygen flask combustion. In Rothchild, S. (Ed.): Advances in Tracer Methodology, New York, N. Y., Plenum Press, 1968, pp. 67-79.

Serial No. NCI-3503
1. Lab. of Chemical Pharmacology
2. Molecular Pharmacology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Pharmacology and Mechanisms of Action of Antitumor Drugs

Previous Serial Number: Same

Principal Investigator: Vincent H. Bono, Jr.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	3
Professional:	1
Other:	2

Project Description:

Objectives:

The objective of this project is to determine the metabolism of new anticancer agents and to study their mechanism of action and the mechanisms of tumors resistance to these agents.

Methods Employed:

Techniques are developed for the isolation and assay of the compounds and their metabolites. Using these chemical methods and/or radioactive labeled materials, the distribution, excretion and metabolism of the compounds are studied in animals and patients. Radioactive tracers are used to determine the effects of drugs on normal enzymatic processes in animals and in vitro systems. Extensive use is made of paper and column chromatography, high voltage paper electrophoresis, autoradiography, liquid scintillation counting and ultraviolet spectrophotometry.

Major Findings:

A study of the reactions of hydroxyurea with ferric and cytochrome C was continued during the past year. This study was initiated because of the findings of Adamson et al. (LCP:NCI) that 5 to 10% of a dose of hydroxyurea was excreted as respiratory CO_2 in rats and mice. Reaction of hydroxyurea with ferric iron and with ferri-cytochrome C had previously been found to produce CO_2.

A more detailed study of the oxidation of hydroxyurea by ferric showed that the initial carbon containing product is cyanate. This subsequently hydrolyses to carbonate which is the source of CO_2.

The interaction of hydroxyurea with cytochrome C showed that here also, the production of cyanate is important. Prolonged incubation of ferricytochrome C with hydroxyurea results in progressive binding of the hydroxyurea carbon to cytochrome C. The bound carbon was found to be present primarily in a single abnormal amino acid, homocitrulline. It is known that cyanate reacts with ε-amino groups of lysine in proteins to produce homocitrulline. It was subsequently found that hydroxyurea hydrolyses to yield cyanate slowly at neutral pH, which in turn reacts with lysine of cytochrome C. The initial interaction of hydroxyurea and ferri-cytochrome C was also found to result in the oxidative conversion of hydroxyurea to cyanate. Thus, the possibility has been suggested by these studies that hydroxyurea may exert its effects as a result of its oxidative conversion to cyanate, at specific sites within a cell, which in turn may react with specific amino or sulfhydryl groups of cellular proteins.

The effects of cyanate on cell growth and nucleic acid synthesis in L1210 cells, in cell culture, were determined. Cyanate totally inhibits cell proliferation after a lag period of 4 hours and if removed from the medium after about one cell cycle time, cell division again resumes. The subsequent growth curve indicates that only a small fraction of the cells were killed and that some synchronization of cell division has been produced. Cyanate inhibits DNA synthesis to a greater extent than RNA synthesis. A concentration of 2.5 mM cyanate inhibits DNA synthesis by 50% while 20 mM cyanate is required to inhibit RNA synthesis by 50%.

Qualitatively, the reversible inhibition of cell proliferation, induction of synchronous cell division, and the selective inhibition of DNA synthesis produced by cyanate are suggestive of hydroxyurea effects. However, the quantitative correlation of these effects is poor and it can be only suggested that hydroxyurea and cyanate have a common action.

The details of inhibition of Novikoff hepatoma ribonucleotide reductase by hydroxyurea were studied. Available evidence indicates that this enzymatic step is the site of action of hydroxyurea in exerting its selective inhibition of DNA synthesis. It was found that 2 mM hydroxyurea inhibited enzymatic activity by 50%. Pre-incubation of the enzyme with hydroxyurea does not increase inhibition and full activity of the enzyme is restored by subsequent dialysis. Kinetic studies indicate that the inhibition appears to be of competitive character with respect to dithioerythritol and of a non-competitive character with respect to uridine diphosphate. These studies support the idea of a specific reversible interaction between HU and the ribonucleotide reductase system. It more specifically suggests that hydroxyurea may interfere with the function of the sulfhydryl reducing substrate.

Significance to Bio-medical Research and the Program of the Institute:

A knowledge of the pharmacology of antitumor agents helps to explain clinical effects and aids in effective clinical use of drugs. It may serve to indicate modifications of dose regimen or modification of the structure of a compound to gain increased antitumor activity.

Proposed Course:

1. Completion of studies in process on the mechanism of action of hydroxyurea.

2. Pursue the use of tissue culture and chemotherapeutic drugs for the investigation of unexplored areas in the mechanism and control of cell division.

3. Pharmacological and biochemical studies of such other new antitumor agents as attract our attention.

Publications:

Kohn, K. W., Bono, V. H., Jr., and Kann, H. E., Jr.: Anthramycin, A New Type of DNA Inhibiting Antibiotic: Reaction with DNA and Effect on Nucleic Acid Synthesis in Mouse Leukemia Cells. Biochemica et Biophysica Acta, 155: 121-129, Jan. 1968.

Serial No. NCI-3511
1. Lab. of Chemical Pharmacology
2. Molecular Pharmacology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Effect of Cancer Chemotherapeutic Agents in Nucleic Acid In Vitro and In Vivo

Previous Serial Number: Same

Principal Investigator: Kurt W. Kohn

Other Investigators: Dr. Herbert E. Kann, Jr., Mr. Carlos Spears, and Dr. Michael Emmer

Cooperating Units: None

Man Years

Total:	3
Professional:	2
Other:	1

Project Description:

Objectives:

To study the structure and function of DNA in relation to the effects of chemotherapeutic agents and the nature of the neoplastic process.

Methods Employed:

1. Liquid scintillation counting.
2. UV-Spectrophotometry (Cary and Gilford recording spectrophotometers.
3. Analytical ultracentrifugation (Beckman Model E).

Major Findings:

I. Reaction of Anthramycin with DNA

Anthramycin is a recently discovered anti-tumor antibiotic that is structurally unrelated to previously known antibiotics. We had previously found that anthramycin inhibits nucleic acid syntheses in mouse leukemia cell culture and that it interacts chemically with DNA. This lead us to believe that the antibiotic probably acts by interfering with DNA template function.

Our major efforts during the past year have been in pursuit of the following questions: (1) What are the chemical features of the interaction with DNA? (2) How does the inhibition of DNA template function lead to cell death?

A) Interaction of anthramycin with purified DNA

An unexpected finding (reported in last year's Annual Report) was that the interaction requires a substantial amount of time. This was unexpected because the structure of anthramycin did not suggest any obvious way in which the antibiotic might react covalently with DNA, whereas previously described antibiotics that bind to DNA non-covalently interact quite rapidly.

A second unusual feature of the interaction was its high degree of specificity for helical DNA. Contrary to previously known DNA-binding agents, there was no detectable reaction with free nucleotides or with RNA.

1) What is responsible for the slowness of the reaction?

There are several a priori possibilities: (1) The antibiotic must be activated to a form that then reacts rapidly. (2) DNA must undergo an improbable conformation change before it can interact with an anthramycin molecule. (3) The accessibility of the binding site is sterically limited. (4) A covalent bond is formed.

The first two possibilities are unlikely in view of the findings that the initial reaction rate depends on both the DNA and the anthramycin concentrations. (The kinetic dependences are not simple and are still under study.) If a unimolecular process involving one of the reactants were rate-limiting, the rate should have been independent of the concentration of the other reactant.

(The starting material for our work is the methyl ether of anthramycin (AME). In these experiments, AME was allowed to hydrolyse prior to mixing with DNA. Hence, any time-lag due to a requirement for a unimolecular activation of AME is avoided.)

The second two possibilities are more difficult to distinguish. Proof of covalent bonding would probably require isolation and characterization of an anthramycin-nucleotide compound. This has thus far not been accomplished. Several observations were made that tend to favor a covalent bond: (1) The DNA complex survived repeated passage through a Sephadex column, and retained a constant ratio of anthramycin to nucleotides. (2) The complex is stable in 0.1N NaOH. In this medium, the secondary structure of DNA is completely disrupted, so that any steric sites should have been destroyed. (3) The complex is not destroyed by Ag^+, even though Ag^+, by altering the secondary structure of DNA, dissociated tight non-covalent binders such as actinomycin and daunamycin. (4) Although free anthramycin was found to be decomposed by heat, NaOH, or Ag^+, it was stable to these conditions when bound to DNA. (This indicates that a labile region of the anthramycin molecule either is sterically protected by the DNA or is actually involved in a covalent bond with the DNA.) (5) The complex was found to dissociate below pH 3, releasing unchanged anthramycin. The decomposition was time dependent, as if a covalent bond was being broken. If the binding were non-covalent, the disruption of DNA secondary structure at low pH should have released the bound molecule almost instantaneously.

2) Reaction kinetics

The mechanism of the reaction of anthramycin with DNA was further investigated by kinetic studies. The reaction rate was found to increase with H^+ concentration (although there was no net consumption of H^+.) This fact will be important when specific reaction mechanisms are considered.

The reaction rate with helical DNA decreased with ionic strength, whereas with denatured DNA the reverse relation was found. This indicates that some special feature of DNA secondary structure is required for reaction.

3) Specificity

The specificity of the reaction was further explored. Experiments with synthetic bihelical deoxypolynucleotides revealed that dGdC reacts, whereas dAT does not react at all. Comparisons between bacterial DNA's of widely differing GC contents, however, failed to show a clear-cut dependence of the reaction rate on %GC. This indicates that the reaction rate depends on more than one base.

B) Effects on RNA Synthesis in Mouse Leukemia Cells and Relation to Cell Lethality

An attempt was made to relate inhibition of RNA synthesis to lethality in L1210 cell culture. Cell lysis and dye permeability were used as measures of cell death. Anthramycin and actinomycin were compared. For a given extent of inhibition of RNA synthesis, anthramycin was generally found to produce greater lethality than did actinomycin. The interpretation was clouded by the fact that the degree of inhibition of RNA synthesis increased with time. Studies as a function of time supported our original finding, but the interpretation of the results is still not entirely free of ambiguity.

Sucrose gradient studies of pulse-labelled RNA synthesized by drug-treated cells showed that actinomycin selectively inhibits the synthesis ribosomal precursor RNA, confirming similar findings reported in the literature for other cell types. Anthramycin, by contrast, had no such selective effect. Instead, the degree of inhibition seemed to increase with the molecular weight of the product. This problem is currently under study.

II. Preliminary Studies on Control of DNA Synthesis

Rationale:
There must be some mechanism, probably humoral, that inhibits the proliferation of normal cells of a given tissue, when that tissue has reached an optimum size. It is a reasonable guess that malignant cells are, for one reason or another, insensitive to this control. If this mechanism were sufficiently understood so that it could be activated artificially, then it might be possible to inhibit temporarily the proliferation of the normal cells of certain critical tissues. Malignant cells, being insensitive to the normal control mechanism, should continue to proliferate, regardless of which of many possible mechanisms is responsible for the malignancy. Known chemotherapeutic agents could then be employed to kill selectively the proliferating malignant cells. The normal cells that are temporarily in a non-proliferating state would be protected. The potential power of this type of therapeutic strategem would be (1) that it may work for all types of malignancies and (2) the problem of drug-resistance may not arise.

The above considerations were our motivation for looking for ways to get at the normal mechanism of DNA synthesis control. Regenerating rat liver was selected as a system for study, since it affords an opportunity to compare cells that are "on" or "off" with respect to DNA replication. Biological considerations strongly suggested that the DNA itself, or the nucleoprotein complex in which it exists in the cell, must have an "on" or "off" state that regulates DNA synthesis. It seemed likely, therefore, that the DNA, as it exists in the isolated nucleus, may be in an "on" or "off" configuration, depending on whether the nuclei were isolated from normal or regenerating liver. Nuclei were therefore isolated from normal and regenerating rat liver and tested for endogenous DNA polymerase activity.

Thus far, only a small number of preliminary experiments have been carried out. The results indicate that certain technical problems must be resolved before this type of study can be carried out successfully. Low levels of DNA polymerase activity were detected both in normal and regenerating liver nuclei. However, there was no clear-cut difference between the two. Probably the most important technical limitation to these studies is the presence of degradative enzymes in the nuclear preparations. We plan to try to circumvent this problem.

Proposed Course:

1) Continue studies of the kinetics and specificity of the reaction of anthramycin with DNA.
2) Extend the RNA synthesis studies to include various drugs that interfere with DNA transcription. The objective would be to determine whether such drugs fall into categories that produce different patterns of RNA synthesis inhibition, and to attempt to relate any such differences to biological effects of these drugs.
3) Explore the possibility of using isolated cell nuclei as a model system for the study of DNA and RNA synthesis inhibition by drugs.

Publications:

Kohn, K. W. and Spears, C. L.: Buoyant density changes and modes of decomposition. Biochim. Biophys. Acta 145: 720-733, 1967.

Kohn, K. W. and Spears, C. L.: Stabilization of nitrogen-mustard alkylations and inter-strand crosslinks in DNA by alkali. Biochim. Biophys. Acta 145: 734-741, 1967.

Kohn, K. W., Bono, V. H., and Kann, H. E.: Anthramycin, a new type of DNA-inhibiting antibiotic: Reaction with DNA and effect on nucleic acid synthesis in mouse leukemia cells. Biochim. Biophys. Acta 155: 121-129, 1968

NATIONAL CANCER INSTITUTE

ANNUAL REPORT

ETIOLOGY

July 1, 1967 through June 30, 1968

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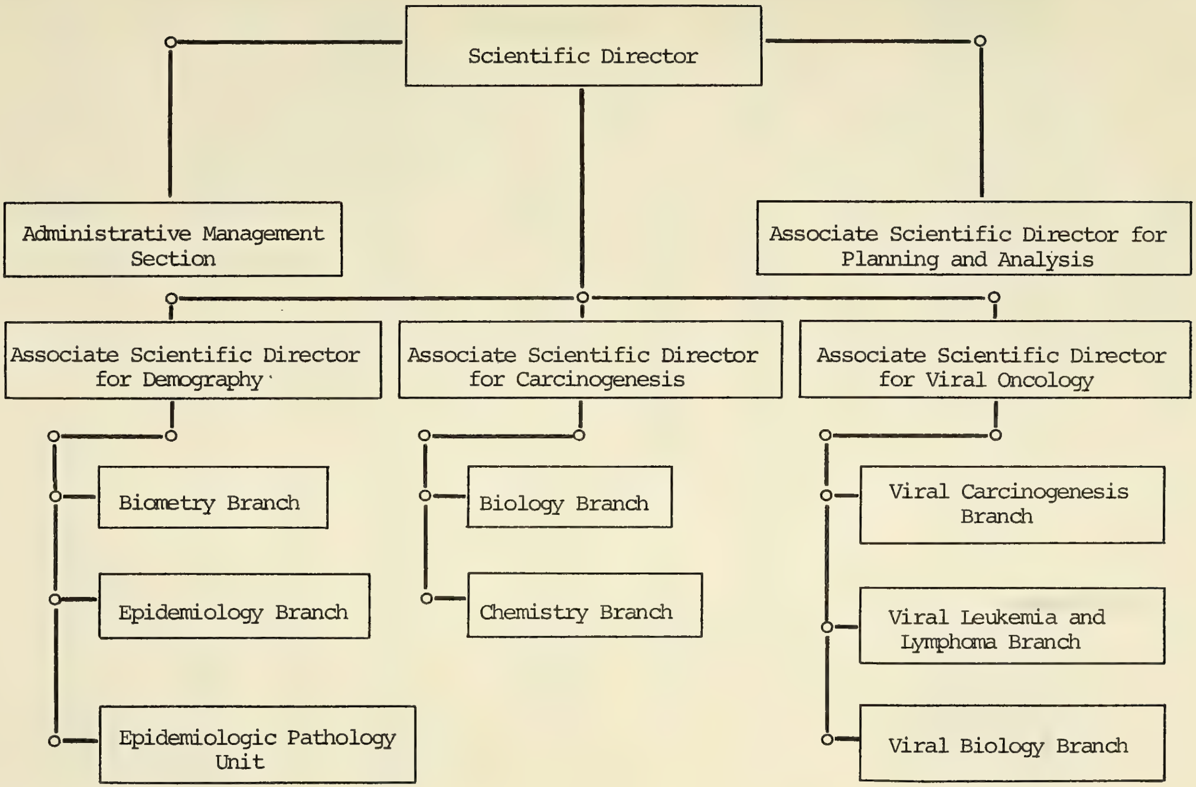
* S.C. -- Service Contracts

CONTRACTS IN PROCESS OF NEGOTIATION AT TIME OF PREPARATION OF THIS REPORT

Contractor	Title	Approximate Amount
Contractor Unknown	Carcinogenesis Abstracts	$ 26,000
Battelle Northwest	Co-Carcinogenicity of Industrial Pollutants and Tobacco Smoke	$ 99,000
Bio-Research Consultants, Inc.	Large Scale Screenings of Environmental Agents	$ 58,000
California State Dept. of Public Health	Roles of DNA and RNA Viruses in Solid Tumors	$172,521
Hine Labs. (proposal A only)	Co-Carcinogenicity of Industrial Pollutants and Tobacco Smoke	$ 44,000
Institute for Medical Research	Studies of Human Milk and Mammary Tumors	$ 35,812
Litton Company	Large Scale Screenings of Environmental Agents	$136,000
Missouri, Univ. of	Virus Transmission Studies in Mini-pigs	$ 60,500
National Academy of Sciences	Preservation of an Index to the Medical Experience of the U.S. Army in World War II	$ 34,310
Pasadena Foundation for Medical Research	Chemical Carcinogenesis on the Cellular Level	$ 35,000
Rutgers University	Immunization and Vaccine Studies in Murine Model Systems	$ 39,790
Smithsonian Institute	Preparation of the Monograph of Marmosets	$ 35,000

Contractor	Title	Approximate Amount
Southern California, Univ. of	A Comprehensive Field and Laboratory Research Program on the Etiology and Epidemiology of Human Cancer	$549,213
Tadanoumi Hospital	Epidemiological Study of Workers Exposed to Mustard Gas	$ 12,000
Weizmann Institute	An Analysis of the Mechanism of Neoplastic Cell Transformation	$ 55,000

Note: Narratives for these new contracts do not appear in this Annual Report.


Scientific Director
Administrative Management Section
Associate Scientific Director for Planning and Analysis
Associate Scientific Director for Demography
Associate Scientific Director for Carcinogenesis
Associate Scientific Director for Viral Oncology
Biometry Branch
Epidemiology Branch
Epidemiologic Pathology Unit
Biology Branch
Chemistry Branch
Viral Carcinogenesis Branch
Viral Leukemia and Lymphoma Branch
Viral Biology Branch

SUMMARY REPORT
ETIOLOGY AREA - NATIONAL CANCER INSTITUTE
July 1, 1967 - June 30, 1968

At the October 9, 1967 Program Review meeting of the National Advisory Cancer Council, the Etiology Area program was presented; at that meeting, the viral oncology aspects were emphasized over the chemical carcinogenesis and biometry and epidemiology aspects. For this review, a statement was prepared by the Scientific Director for Etiology on several aspects of cancer causation and prevention and on the Etiology Area program. It seems appropriate to reproduce this statement as part of the Annual Report:

GENERAL ASPECTS OF THE ETIOLOGY PROGRAM

by

Carl G. Baker, M.D.
Scientific Director for Etiology, NCI

> *"Organism and environment form a whole and must be viewed as such."*
> *--Starling*

> *"It is surely time that we should drop the medieval concepts concerning causation, and think in terms of multiple correlation."*
> *--J. Huxley*

I. Goal and Objectives. The over-all goal of the Etiology program is, through research, to provide as rapidly and effectively as feasible within the existing resources and constraints the means for preventing cancers in as many people as possible.

   A. The broad objectives selected in conjunction with this goal are those of the three major organizational segments within the Etiology Area:

      1. Viral Oncology - to determine the significance of viruses in the induction of cancers in man and develop means for preventing these cancers with virological and immunological techniques.

      2. Chemical Carcinogenesis - to determine the significance of chemical agents in the induction of cancers in man and develop means for preventing these cancers with modification of exposures to identified agents and possibly with protection of individuals potentially or actually exposed to such agents by alteration of body functions.

3. Biometry and Epidemiology[1] - to provide a continual monitoring of populations for cancer incidence, prevalence, and mortality; to identify groups with different risks to cancers and seek associated internal and external environmental and genetic factors; to conduct observational research in situations where society or nature has provided the experiment on cancer such as studies of occupational groups, migrant populations, and groups with other diseases, etc.; and to collaborate in a variety of investigations requiring epidemiological, demographic, statistical, and mathematical expertise.

B. Coordination and integration of various aspects of these three organizational segments is likewise a continuing, required objective.

1. Coupling is essential between observational investigations identifying groups with different cancer risks and possible associated factors and experimental laboratory investigations concerned with identifying carcinogenic agents, their modes of actions, means of modifying their cancer-inducing capabilities, etc.

2. Increasing attention is being given to combinations of agents involved in carcinogenesis, including viruses and chemicals (carcinogenic and other).

C. Because chemical carcinogens often display a universality of action (i.e., many chemical agents produce cancers in several organ sites and tissues), much of the Etiology program is not geared to research on cancers of a particular anatomical site or histologic type, but rather to research on identification and characterization of special hazards and on efforts to understand the processes of carcinogenesis in general.

D. Although a wide variety of experimental investigations in animals is open to investigators of carcinogenesis, because it is inappropriate to evaluate directly carcinogenic agents in man, studies in man are limited primarily to observational investigations; from this follows the suggestion that much of the knowledge of carcinogenesis in man must be derived from animal studies; however, the relationships between animal responses and those in man are not clear and, without the availability of experimental studies in man, most cannot be related with hard data (e.g., as with the relationship of clinical data from the well-designed therapeutic trial of chemotherapy and the animal screen data).

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[1] The Biometry and Epidemiology Branches are also directly concerned with several aspects of diagnosis and therapy and have made major contributions to design of therapeutic trials and other experimental efforts, evaluation of end results of therapies, evaluation of possible diagnostic and detection studies, etc. These Branches could as logically be placed organizationally within the Office of the Director, NCI.

E. Cancers are chronic diseases; from this follows several difficulties.

1. Observational and experimental investigations in carcinogenesis are long-term and expensive in resources.

2. The long-term and resource-consuming nature of carcinogenesis investigation, with fewer resulting publications and often with negative findings following long effort, discourages many investigators from undertaking carcinogenesis studies.

3. A major part of a carcinogenesis program is the collection of test data for carcinogenicity; this activity requires long-term, routinized, and repetitive efforts, viewed rather unfavorably by investigators and reviewers alike.

4. Effects of decisions cannot be evaluated before considerable time has elapsed, making sound decision-making more difficult.

5. This extended time-frame and the slowness in relating results to investments make difficult the maintenance of program enthusiasm, momentum, and long-term support.

II. Concepts of Cancer. Program decisions are affected by concepts held as to the nature of cancers, the processes of their induction, and the factors involved in transformation into malignancy.

A. The concepts are developed from a composite of viewpoints gained by:

1. Considering living systems from different organismic, hierarchical levels (hierarchies of processes and of structures): populations of individuals; individual organisms; organs and tissues; cells; and subcellular components, including the molecular level -- all in relation to different time-frames;

2. Applying knowledge (conceptual and informational) from the standpoints of various scientific disciplines and medical specialties;

3. More subtly, employing varied classifications for organizing information about organisms as derived from various disciplines and specialties, especially in reference to cancer; and

4. Taking into account other information, especially incidence data, on cancers such as:

   a. Associations between various (chemical, physical, and biological) agents and cancers [Tables 1-6] [q.v.] (1-4);

   b. Sex differences [Figure I, Table 7] (5-7);

   c. Geographic and regional differences [Figure II] (5-7);

   d. Racial differences [Figure III] (5-7);

    e. Associations between cancers and other biomedical disorders (8-9);

    f. Trends [Figure IV];

    g. Organ site and tissue differences [Table 8]; and

    h. Species and strain differences [Table 9] (10).

B. It is important to examine periodically concepts held and the assumptions and data underlying them, not only to up-date the concepts in the light of new data, but also to attempt to escape from tradition and to consider a variety of conceptual schemes with different purposes in mind.

C. There appears to be some advantage to focusing conceptually upon one of the mid-organismic levels -- the cellular level -- since: (1) cancers (determinable as malignant neoplasms only at the higher levels of tissues, organ sites, individuals, and populations) can be considered to be derived from modified cells; and (2) these cell modifications can be conceptually handled as being produced by changes at that level (impingement on the cell by several chemical, biological, and physical factors, including interactions among cells) or at lower levels (modifications of cell components conceptualized in molecular biology terms or at the next level in terms of cell physiology).

    1. In addition, genetic information brought to cells through heredity (both in terms of the pool of genes and of modifications produced by environmental impingement prior to birth of individuals) must be considered in concepts of cancers.

D. From current molecular biology dogma of cell control mechanisms (11) cancer initiation can be conceptualized as modification at any of several points in the system[2] representing a cell [schematically shown in Figure V] (12).

    1. The "points" may be structural modifications (structures are slow processes of long duration) or functional modifications (functions are quick processes of short duration).

        a. Functional modifications can result from a change in traffic flows along metabolic pathways without any necessary alteration observable in morphological structures nor isolatable as structural entities; in other words, a system with one set of rate phenomena and a particular pattern of behavior can change to one with a different set of rate phenomena and a new pattern of behavior without observable structural changes; moreover, both systems can be relatively stable and, in the case of biological systems, either can be transmitted from parent to daughter cells[3].

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[2] Herein meaning a complex of elements in mutual interactions.

2. Structural and/or functional modifications can result from impingement on cells by a variety of factors [Figure V], singly or in combination, frequently or rarely, interacting readily or only under special conditions.

    a. These factors can come from the external environment or the internal environment of the host individual.

    b. Factors reaching the cell may have been indirectly derived from the external environment through modification of an environmental agent by processes in the host individual or by interaction with other factors.

    c. At least under some conditions, the number of tumors found following administration of small amounts of a carcinogenic agent can be considerably increased if another agent, itself not carcinogenic, is subsequently administered; reversal of the order of administration may give no tumors; such findings suggest that modifications leading to cancer can be grouped into two phases: initiation and promotion, and different agents may be initiating or promoting (20).

3. Structural and/or functional features of cells may also be considered as modifications from those in precursor cells (e.g., the Philadelphia chromosome in chronic myelogenous leukemia) or as modifications when compared to the same types of features in cells of the individual's parents, either as direct differences (e.g., in cancers associated with genetic anomalies such as higher leukemia risks among mongoloids) or as indirect differences (e.g., different cancer susceptibilities to particular chemical or viral agents).

4. With the very large numbers of interactions taking place within non-dividing or dividing cells over the life-time of an individual, even without regard to interactions with impinging factors, the opportunities for modifications of structural or functional features should be great; or, to put it another way, the chance of errors in cell control information transcription, translation, expression, and feedback should be high in the face of so many reactions[4].

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[3] Such considerations suggest the need for additional data on rate phenomena in complex biologic systems (in cancers and in controls), perhaps with the aid of complex systems models (13-19).

[4] It would be important to know the rate of error production in dividing and non-dividing cells and in individual organisms and what proportion are expressible as cancers. Is the rate of production of cells potentially expressible as cancers low or is it high but the transformed cells do not find fertile soil and never show up as malignant neoplasms? Can means be devised for quantifying these rates?

    a. Certain patterns of such modifications lead to cell behaviors culminating in cancers (the 16 percent of deaths with cancers presumably bears some relation to the frequency of these patterns showing up as cancers, rather than as some other disorder).

    b. With so many opportunities for errors in cell control information fidelity, it is perhaps surprising that we do not see more cancers, though the total number of cancers in a population are distributed more heavily in the older age groups (about one-half the cancer deaths occur beyond the age of 65 years); we have to explain why there are not more cancers than we see.

5. It seems reasonable to conclude that interactions of cells with impinging factors lead to increased rates of modifications, some of which culminate in cancers; e.g., interactions with certain chemicals or viruses lead to increased numbers of cancers in individual organisms.

    a. The sites of interactions are several: DNA; mRNA; tRNA; ribosomes; polysomes; enzyme and structural proteins; cell membranes; etc. (i.e., the "points" referred to earlier at II, D, 1 in reference to Figure V).

    b. One difficulty in research on cancer causation is likely not the problem of finding "the cause" of cancer among many elements of great complication, but rather the problem of determining the significance of many interactions within systems of great complexity.

III. Broad Program in Cancer Etiology and Prevention. The conceptualization of cancer as delineated above provides a basis for formulating a broad program in cancer etiology and prevention and suggests many points of attack.

A. However, in program development, implementation, and monitoring, it is important to shift viewpoints and consider the ends to be attained; one of the main functions of a well-developed, broad-scale program is to attain defined ends by linking them to the conceptual base by means of several streams of various research efforts, integrated into a program whole, which in turn assists in making decisions on priorities and in assessing progress for the total program.

1. Moreover, clear definition of ends and sound formulation of program should provide a definitive basis for specifying the kinds of data needed to answer crucial questions, the sources of the data, correlations needed with other data, and the use to which the derived information is to be put; the importance of the data provides further inputs for making priority decisions on allocations of resources and program emphases.

2. It should be noted that considerations at the broad program level also require a shift of emphasis to a higher organismic level -- the individual organism -- since the program focus is cancer etiology and prevention, and evaluation of program results must ultimately be in terms of success of preventing cancers; cancers are definable as disorders in individual organisms, not at lower organismic levels.

3. It should not need emphasizing that the important individual organism is man; all efforts in the program should be weighed as to the likelihood of assisting in preventing cancers in man and other aspects subordinated to this cardinal consideration.

   a. Results from studies on populations of individuals provide further basis for program priority determinations and emphases.

   b. Obviously, due attention must be applied to the long-term time-frame demanded by the nature of carcinogenesis, requiring balance between short- and long-term objectives.

B. The main elements of a broad program on the etiology and prevention of cancer is sketched in Figure VI.

1. Further elaboration with indications of areas of research efforts in relation to the main elements will be discussed.

2. The reports of the Associate Scientific Directors and Branch Chiefs provide further information on the respective areas, including indications of relationships to a broad effort in cancer etiology and prevention.

3. A hierarchy of research efforts is required in a broad program, with some efforts of immediate and direct relevance to the central theme, others less so, and still others of long-term potential with considerable degrees of uncertainty (in actual research, the nature of the total effort is a spectrum, not a hierarchy).

4. Various activities in the major organizational segments of the Etiology Area can be related to program areas in Figure VI.

   a. For example, the Biometry and Epidemiology Branches' areas of responsibility include three major blocks on the chart: (1) Characterization of Population Groups with Different Risks to Cancers; (2) Prospective Studies; and (3) End Results Studies.

   b. It is also of interest to trace through the chart the viral and the chemical carcinogenesis efforts and note which major blocks of the central theme are most pertinent to respective program efforts; e.g., both areas are tied to the major block, Identification of Cancer-Inducing Agents, but in terms of preventive actions the virological efforts are most likely to be in relation to the block, Modification of Members of Groups with Different Risks to Cancers (e.g., by immunologic means), while in the case of chemical carcinogenesis, the preventive measures will more likely relate to the block, Elimination of Exposures to Cancer-Inducing Agents (e.g., elimination of the material involved in an occupational process).

5. Additional relationships will be discussed.

C. There appears to be a widespread view that with introduction into the activities in our society of large numbers of agents each year, these agents should be evaluated as to their hazard for man, including the ability to produce human cancers; pursuit of how this is to be done illustrates insufficient integration of activities of different specialties (e.g., epidemiology and chemical and biological aspects of screening), the incompleteness of specifying program and data needs, and the severe difficulties in carcinogenesis research, in part because of the long-term nature of the process and the great complexity involving interaction of many factors.

    1. What is to be done to clarify the relationships between animal data on chemical carcinogenesis and significance for human cancers?

    2. How can data on chemical carcinogenesis be systematically compiled so that data from different investigative groups can be brought together meaningfully?

        a. Even for a single compound, large numbers of variables need to be taken into account for evaluation of carcinogenicity: species and strain; types of cancers, including "spontaneous" rates; dietary conditions and state of health and viral experience of the organism; physical and chemical attributes of materials to be evaluated (e.g., degree of purity of chemical compounds); the routes of administration; vehicle carrying the test material; site of administration; dose including amounts and scheduling of administration; reactions of the test system in addition to cancer formation (e.g., short- and long-term toxicity reactions); endpoints or scores used for determining the significance of the evaluative studies (e.g., number of tumors, latent periods, type of histology of tumors); etc.

        b. An initial step, which the staff is beginning, is an orderly comparison of the data from various animal studies on those agents for which there is wide consensus that they are carcinogens for man; the large number of variables mentioned above plus the difficulty of combining the data collected under a variety of approaches makes even this task a formidable one.

        c. If correlations of patterns of data from the animal studies can be seen in relation to those agents carcinogenic for man, a basis for a more systematic screen may be in hand.

3. What are the best approaches to be used in evaluating the carcinogenic effects of multiple factors interacting at various dose levels and over differing periods of time?

    a. Though the number of variables are large for evaluation of single compounds as to carcinogenicity, they rise geometrically when combinations of agents are involved.

    b. Much more systematized information and data are needed on dose relationships in carcinogenesis (21-22).

    c. Because of the great complexity, the problem may need to be approached as certain systems engineers approach complex situations: "the black box" approach where the complex arrangements in the box are never determined directly, but indirectly by determining input and output relationships (19, 23).

D. These and other issues are in need of further clarification as part of the etiology and prevention program for chemical carcinogenesis, and additional planning will be done as feasibility develops (see Figure VI, the major block, Identification of Cancer-Inducing Agents; for comparison in the Viral Oncology program area, see Program Plans, primarily Phases I and II) (24).

E. Similar treatment is needed for other program areas such as Characterization of Groups as to Structural and Functional Parameters in the major block, Characterization of Population Groups with Different Risks to Cancers [Figure VI].

1. An example here concerns the general question as to differences between those who get cancers and those who do not; e.g., how do the characteristics of the large numbers of heavy smokers who do not get lung cancers differ from those of the fewer heavy smokers who do? Or from those who do not smoke but get lung cancer? What characteristics should be studied? [5]

2. Again, to deal with such issues, combinations of efforts from different specialties are required (epidemiologic concepts and techniques for identifying groups with different risks to cancers and laboratory sciences concepts and techniques such as immunology or electron microscopy for determining certain functional and/or structural parameters); and the data from the different specialty areas must be brought together in a coordinated manner and correlations sought.

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[5] In England between 1911-1940 about one-half million workers (cotton mule spinners; chimney sweeps; gas, tar, pitch and creosote workers; fishermen, boatmen, fishing-net workers; and engine and crane drivers and firemen) were constantly in contact with external agents presumed to be more highly carcinogenic than those to which most of the remainder of the population was exposed; yet, of this one-half million, only 723 or slightly over 0.1% developed scrotal skin cancer.

F. Such considerations reemphasize the statement in III, A, 1 and point up the requirement of good judgment in defining objectives and selecting significant structural and/or functional parameters on which data should be collected for correlation with data on cancer-risks groups.

IV. Magnitude of Task and Investments Required. The magnitude of the task outlined as a program in etiology and prevention and the investment costs required for its pursuit do not appear to be adequately appreciated beyond a few individuals.

A. In addition to the above complexities and difficulties, other problems include:

1. The rate of introduction of agents into activities of society far exceeds the rate at which agents can be evaluated (at least with current capacities) for carcinogenicity (even in animal systems, to say nothing about significance for man).

a. One can suppose that short cuts (less lengthy evaluative tests) to assessing agents may be developed in the future, but these must be validated against direct definitive information from animal systems that can be shown to have relevance in man before the short cuts can be used.

2. The costs in dollars and other resources (including space and construction) for implementing efforts in cancer etiology and prevention exceed those for many other areas of biomedical research.[6]

3. Instituting preventive measures often require unusual cooperation among many groups, at times with the aid of complex legislative and control measures, sometimes in the face of lack of absolute "proof" of hazard.[6]

B. To accomplish many aspects of a broad program on carcinogenesis, especially to provide requisite data on tests for carcinogenicity, modification is required in the present system of research in biomedicine, not only as to order of magnitude of dollar investment, but also the over-all approach (25, 26).

1. Industrial capability must be joined more effectively in cancer research.

2. Emphasis on circumscribed projects rather than broader segments of research efforts is too costly in research program management time, efforts, and energies (25).

V. Program Emphases. The reports of the program leaders of the Etiology Area provide considerable information on activities within the respective areas (q.v.), and, because of the special review in the Viral Oncology segment scheduled for the October National Advisory Council meeting, the reports from this segment are particularly extensive.

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[6] Compare plans for the Lung Cancer Task Force.

A. In addition, several tables and charts under Tab II provide additional information on program emphases.

   1. The first chart (II-A) and table (II-1) give distribution of fiscal year 1967 funds in the Etiology Area.

      a. Viral oncology efforts account for 64 percent of the funds; the Special Virus-Leukemia Program portion accounts for 50 percent of the funds.

      b. Chemical carcinogenesis efforts account for nearly one-quarter of the funds.

      c. Biometry and epidemiology efforts account for 10 percent of the funds.

   2. Chart II-B indicates the distribution of funds for the Biometry Branch activities.

      a. Prevention: 35 percent; treatment: 22 percent.

      b. Diagnosis: 35 percent, most of which is accounted for by a long-term evaluation of mammography with the Health Insurance Plan of Greater New York.

   3. Chart II-C indicates the distribution of funds for the Epidemiology Branch activities.

      a. Occupational cancer studies: 26 percent.

      b. Childhood cancers: 32 percent; adult cancers (other than occupational cancers): 30 percent.

      c. Veterinary cancer: 12 percent.

   4. Charts II-D and II-E indicate the distribution of contract funds for carcinogenesis studies (about 80 percent of the total carcinogenesis dollars).

      a. About 55 percent of contract funds are for identifying carcinogenic hazards and 45 percent for studies on mechanisms of carcinogenesis.

      b. Of the $2.8 million in contracts on hazard studies, percentages on types of hazards under study are: pesticides: 17 percent; drugs: 11 percent; occupational hazards: 16 percent; tobacco: 10 percent; air pollution: 9 percent; food contamination: 17 percent; and other environmental agents: 20 percent.

      c. These same contracts for study of hazards may also be classified (Chart II-F) into: bioassay: 46 percent; screening: 9 percent; chemistry: 30 percent; and histopathology: 15 percent.

   5. Chart I and Table I indicate distributions for the viral oncology efforts (see additional information in the reports from this program segment).

a. Human leukemia etiology and prevention activities: 23 percent.

b. Special animal ecology studies: 13 percent.

c. Solid tumor virus studies: 21 percent.

d. Program resources and logistics: 23 percent.

e. Biohazards control and containment: 9 percent.

B. Major items of change in emphasis antitipated during the coming year are:

1. The ten-cities epidemiologic survey designed to obtain cancer incidence and other data in conjunction with the next U. S. census will be initiated.

2. Carcinogenesis studies on pesticides will be reduced and those on tobacco, drugs, and screening will be increased.

3. Viral oncology efforts on solid tumor viruses will be increased and investments in some resources production will be decreased.

C. With the possible exception of funding tobacco and smoking research, the above changes will likely, necessarily be accomplished within the approximate levels of current funding.

1. The most serious dollar shortages in terms of need and opportunities are in the chemical carcinogenesis area, particularly testing; these shortages are likely to continue.

2. The need for additional efforts in biometry and epidemiology continues; here manpower shortages are most critical and increasing attention must be given to this problem area over the next few years (training programs in the general area of biometry and epidemiology are not meeting satisfactorily the needs of the cancer field).

3. Within the area of viral oncology, the increased efforts in DNA solid tumor virus research will be funded by reduction in efforts not having as high priorities (this subject will be discussed further during the review of the viral oncology program segment).

VI. Program Management. The organizational structure of the Etiology Area is shown in Figure VII.

A. There are no plans to change the basic structure with its three major segments: (1) Viral Oncology; (2) Chemical Carcinogenesis; and (3) Biometry and Epidemiology.

1. At a later time, it may be important to add another group in chemical carcinogenesis to provide further emphasis on testing, screening, and bioassay.

2. The Associate Scientific Director for Demography position is currently unfilled and the Associate Scientific Director for Carcinogenesis will move to the Division of Environmental Health Sciences around the end of the current fiscal year.

B. Plans are under way to modify program and contract reviews.

1. The present Etiology Contract Review Committee, which has been reviewing all contracts except those funded from the Special Virus-Leukemia Program budget, will be discontinued in the near future.

2. Those contracts concerned with viral oncology, including the resources contracts, will now be reviewed in the system established for review of the Special Virus-Leukemia Program contracts.

   a. In the past, six working groups composed of NCI staff and other scientists have been concerned with six operating areas of the Special Virus-Leukemia Program and have provided initial review of individual contracts; an additional group has been formed to review those virology and resources contracts formerly reviewed by the Etiology Contract Review Committee (Solid Tumor-Virus Group).

   b. Similar committees will be established for: (1) Chemical Carcinogenesis; and (2) Biometry and Epidemiology.

3. The senior program leaders of the Etiology Area will constitute a strategy group for the Etiology Area and will perform the second stage of dual review of individual contracts.

4. A Program Advisory Committee, consisting of non-governmental scientists, is being formed and will work in conjunction with the strategy group and provide advice to the Scientific Director for Etiology.

5. The Etiology Area will continue to obtain advice from many outside investigators on various subjects and for the conduct of site visits.

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The major changes that have occurred in the Etiology Area since last year are: (1) In the Biometry and Epidemiology areas, the Third National Cancer Survey, the first such survey on cancer incidence in 20 years, has been initiated, and studies on migrant and selected population groups now contain more definitive quantitative pathology data; (2) The Viral Oncology area has had increased efforts in the Solid Tumor-Virus area and some reduction in the Special Virus-Leukemia Program (about $3 million of efforts in the SVLP were terminated because of completion of studies, discontinued need for certain types of data due to success of other projects, termination for less than satisfactory performance, and discontinuation of needed projects but of lower priorities in comparison with new needs); and (3) The chemical Carcinogenesis area has had a slight reduction in studies on pesticides (most of which were negative for carcinogenicity) and an increase in screening efforts (assuming new contracts to be initiated late in the fiscal year are actually funded). The major findings from each area are summarized in the reports of the Associate Scientific Directors and Branch Chiefs and further details are given in the individual reports.

No major organizational changes were made during the past year, and, in view of Dr. Falk's departure to the Division of Environmental Health Sciences at the end of the fiscal year, major attention was given to finding his successor. Dr. Umberto Saffiotti, who has conducted extensive carcinogenesis research at Chicago Medical School and in Italy, is joining the NCI staff in mid-May as Dr. Falk's successor. It is anticipated that intensive efforts will be spent initially with Dr. Saffiotti on program planning for the chemical Carcinogenesis area. It is expected that additional efforts will be needed in bringing together in a systematic fashion the technical data in chemical carcinogenesis, particularly in respect to the identification of carcinogenic agents and the test systems used for this purpose.

It is anticipated that the biometry and epidemiology activities will continue essentially along the same lines as the past year, plus the addition of the National Cancer Survey activities. Many needs and opportunities for additional biometry and epidemiology studies in cancer exist, but resources available and manpower shortages in this area will not allow additional efforts in this direction. It is being urged that the extramural training programs of NCI give special attention to this shortage area.

From advances in both RNA and DNA oncogenic viruses research and from the managerial standpoint, it appears advantageous to blend the Special Virus-Leukemia Program efforts and the Solid Tumor-Virus activities into one program. (Though from the standpoint of budget review, especially at the Congressional level, this perhaps is less effective than the separate presentations.) The addition of Dr. Huebner's group and the establishment of field operations in a defined urban population (again assuming contracts initiated late in the fiscal year are funded) greatly strengthens the capabilities in viral oncology, as these activities complement the already strong program in viral oncology in NCI. Developments in the viral oncology area have moved well in studies on Burkitt lymphoma and the herpes-type virus and in the development of methodologies suitable for surveying populations for evidence of previous or current oncogenic virus exposure.

Studies with the type-C virus in leukemia continue to yield little success in establishing biological activity of the virus, despite very extensive efforts to date in several systems by many investigators. This difficulty may be accounted for by the low amounts of virus present in man, and special studies are under way with the known animal model systems for approaching the problem more analogously to the human situation using very low virus dose levels.

The Etiology Area staff is eagerly looking forward to moving into new laboratory space in the fall. Their eagerness is understandable since many staff members have been in crowded, makeshift laboratory space in rented facilities away from the reservation during their initial few years at NIH. The Etiology Area is appreciative of the many people's efforts that have gone into making possible this move.

Three problems continue to exist. Sound project officer management of contracts is sometimes difficult to obtain in a few cases, and the amount of time spent by project officers and senior scientific program management staff on documentation and review seems to be heavy and increasing. The number of proposals on competitive bidding in some instances is excessive in terms of time spent on reviewing by senior scientific staff. The NCI needs to develop a system, perhaps analogous to that of the Department of Defense, whereby senior scientific staff reviews fewer numbers of proposals in selecting the successful bidder.

The Etiology Area, through its Program Management Group, has been conducting program reviews of the main segments of the Area. In addition to the review of ongoing efforts as to their relationship to program objectives, the program reviews also serve to inform each program leader of the objectives, activities, and problems in each other's area. This is particularly important for developing collaborative studies, and, in addition, will likely be needed in the future for defining investigations on cancer causation and prevention when both chemicals and viruses are involved and for meeting the need to relate this kind of information to populations of different risks to the various types of cancer.

The Etiology Program Advisory Committee is being established and the following individuals are being invited to serve: Dr. Richard Mason (Chairman), American Cancer Society; Dr. René Dubos, Rockefeller University; Mr. Jerome Cornfield, University of Pittsburgh; Dr. Maureen Henderson, University of Maryland; Dr. Harold Rusch, University of Wisconsin; Dr. Michael Shimkin, Temple University; Dr. Karl Habel, Scripps Clinic and Research Foundation; and Dr. Charles Evans, University of Washington.

Summary Statement
OFFICE OF THE ACTING ASSOCIATE SCIENTIFIC DIRECTOR FOR DEMOGRAPHY, ETIOLOGY
July 1, 1967 - June 30, 1968

The Demography area consists of three parts: (1) the Epidemiology Branch; (2) the Biometry Branch; and (3) the Epidemiologic Pathology Unit. The objectives of the Demography area include the collection and analysis of demographic and epidemiologic data; end results evaluation of treatment; evaluation of disease definitions, classifications, prognostic factors, and models of therapy; testing of several hypotheses and models for cancer etiology; development of techniques and methodologies in demography, statistics and mathematical statistics; and training in these areas. The staff also provides consultation and collaboration with other members of the staff, the Food and Drug Administration, Regional Medical Program and others, including consultation by a newly established small staff group on computer applications.

The staff of the Epidemiology Branch continues to produce large numbers of elegant studies in epidemiology -- studies based on medical knowledge and experience and on awareness and use of resources primarily available at the national or international level. The Branch took steps to establish a registry of death certificates on children who died of cancer in the United States since 1960. This resource has already proved highly valuable in providing data for a number of studies. For example, among 7,000 Maryland children immunized against measles in 1961 with a vaccine containing viruses capable of inducing cancer in lower species, none of the vaccinated children had died of cancer through 1964. The continuing follow-up of 700 children who as newborns received oral polio vaccine containing SV-40 still reveals no deaths from cancer among the 11 deaths so far reported from the original group. Another study resulting from availability of information from the registry indicates that neuroblastoma in children originates largely *in utero* and varies over time in certain subdivisions of the United States in a fashion suggesting an environmental influence on its occurrence. Other studies include those employing data from the registry and show several associations between cancer of childhood and specific congenital defects. Data from the registry also confirmed the high probability (1 in 5) that the coidentical twin will develop leukemia shortly after the other twin has developed leukemia at an early age. Also reported upon was a study of persons with exceptionally high risk of leukemia; estimates of risk are summarized in the following table.

GROUPS AT EXCEPTIONALLY HIGH RISK OF LEUKEMIA

Group	Approximate risk	Time interval
Identical twins of children with leukemia	1 in 5[a]	Weeks or months
Radiation-treated polycythemia vera	1 in 6	10-15 years
Bloom's syndrome	1 in 8[b]	<30 years of age
Hiroshima survivors who were within 1000 meters of the hypocenter	1 in 60	12 years
Down's syndrome	1 in 95	<10 years of age
Radiation-treated patients with ankylosing spondylitis	1 in 270	15 years
Sibs of leukemic children	1 in 720	10 years
U. S. Caucasian children < 15 years of age	1 in 2880	10 years

[a] Of 22 identical twins with leukemia, the co-twin was affected in 5 instances.

[b] Three leukemics among 23 persons with Bloom's syndrome.

Currently under study are neonatal deaths from congenital cancer, of which there were 130 in the United States from 1960-1964. As compared with type specific cancer deaths under five years of age, there was among the congenital neoplasms a disproportionate number of neuroblastomas, primarily liver cancers and malignant teratomas.

The Epidemiology Branch is also making good use of data available from the military and the Veterans Administration. Using the prospective approach, certain diseases, such as mononucleosis or hepatitis among soldiers in World War II, are being studied for their relationship to subsequent cancer experience. In the retrospective component of the studies, about 3,000 army veterans dying of cancer have been identified, and relationships between cancer and such events as yellow fever vaccination and blood transfusion are being sought.

Nearing completion is the study of the causes of death among 8,047 smelter workers. The results indicate an excess of lung cancer, apparently related primarily to arsenic exposure. Studies in Japan have revealed a marked excess of oral and respiratory-tract cancers and excesses of cancer of the liver, esophagus and skin among former workers engaged in the manufacture of mustard gas. Under analysis are 4,000 deaths among the members of the American Chemical Society, 1948-1967. Clues to chemical carcinogenesis in man are being sought in this study. The system for making standard observations of disease among animals on a mass-scale and permitting computer processing and retrieval earlier developed by the Epizootiology Section is now working smoothly, and data are being collected from several schools of veterinary medicine. One study resulting from such data has shown that the frequency of lymphoma in the boxer breed is about six times greater than in other purebred dogs.

The Biometry Branch continues to provide important information from the end results activities, and again at the next National Cancer Conference summaries of end results for the various sites of cancer will be presented. Trends from these studies include the following: (1) Cancer of the colon is increasing, but is balanced by improved survival; (2) The stable mortality of breast cancer continues and is accounted for by increased incidence in younger women balanced by an increased survival; (3) The increase in survival in acute lymphocytic leukemia found in those institutions giving specialized therapy for the disease is also evident, but to a lesser degree, in other hospitals; (4) An increase in survival of many types of cancer has been demonstrated during the 1950 decade as compared with the 1940 decade; gains in the 1960 decade are evident but are not as large as those achieved earlier; (5) Lung cancer incidence continues to increase; survival, especially in the localized form, has also increased.

More attention is being given to pathology aspects of the end results studies and in the migrant population investigations through cooperation with Dr. Berg in the Epidemiology Pathology Unit. This is permitting additional yields from the end results data in studies important for definition of disease in individuals, classification of diseases, prognostic factors, and evaluation of therapy. Having established a predominance of environmental factors over genetic factors in association with race in the Japanese migrant studies, this study continues with fuller exploration of environmental factors among the Japanese populations, with particular emphasis on different dietary habits in Japan, Hawaii, and the western United States.

The initial planning for the 3rd National Cancer Survey is well along, and the pretest in Birmingham is starting. These surveys, the last one which was conducted 20 years ago, provide the main information on cancer incidence in addition to other important baseline information on cancer demography. The 3rd survey will be conducted in ten metropolitan areas plus two states, surveying a population base of over 22 million people with an expectation of more than a quarter million cancer patients over a three-year period, 1969-1971. These data will be tied to the 1970 census.

The study evaluating mammography as a diagnostic tool for breast cancer indicates that additional cases of breast cancer can be identified with mammography over the older conventional medical examination of breasts. The study will continue for sometime since the primary purpose is to determine whether breast cancer mortality will be affected by this diagnostic procedure. In conjunction with the Breast Cancer Task Force, studies on hormonal levels are being determined in the Health Insurance Plan population of women for the purpose of seeking correlations with different forms of breast cancer. With the VA, a study of prostatic cancer therapy revealed higher rates of cardiovascular deaths in those patients receiving stilbestrol.

Utilizing techniques derived from the earlier development of a model for breast cancer, the staff has been able to forecast survival of lung cancer patients in a controlled clinical trial. A two stage mathematical model of breast cancer has also been developed, and several new developments in mathematical statistics were achieved. Important collaborative studies included investigations in immunology, pattern recognition, and clustering of diseases in time and space.

The Epidemiologic Pathology Unit, working closely with the Biometry Branch, is making important additions to a number of studies by introducing quantitative pathology parameters into many of the studies. This effort includes the standardization of pathology nomenclature and criteria among various investigators participating in migrant and other studies. Particular attention is being given to pathological changes that may be important as precancerous lesions. As part of the effort to improve common criteria, a symposium, "Comparative Morphology of Hematopoietic Neoplasms" was jointly sponsored with the Armed Forces Institute of Pathology. A systematic review was made of hemic neoplasms of invertebrates and lower vertebrates, avians and mammals, including man. Almost all spontaneous hemic neoplasms of the mammals which have been studied are of lymphoid origin; myeloid forms of leukemia are rare. The spectrum of histologic types of lymphomas, a majority of which are nonleukemic, are similar to those which have been observed in man. Little knowledge is available concerning prevalence and morphologic types of hemic neoplasms of subhuman primates; to date, there have been only 21 reports.

An example of the added information made available by quantitative pathology in conjunction with migrant studies is the finding of differences in cancer of the lower colon among Japanese in Hawaii. In Hawaii the cancer occurs usually in the sigmoid, a disease rarely seen in Japan where the difference in location is only a matter of a few centimeters. Additional attention to quantitative pathology information can provide additional clues to the etiology of human cancer. In a study on multiple cancers, 793 cancers were expected in organ systems other than that of the first cancer; 1,643 such cancers were found. Unusually high associations were found between certain types of cancers, such as breast and salivary gland cancers.

The staff and activities of the Demography area constitute a major training resource in cancer epidemiology and associated methodologies. Recruitment of physicians into the Epidemiology Branch has been highly successful, but it is much more difficult to recruit in the Biometry Branch programs where competition and opportunities are high outside the Federal Government. Loss of senior staff in the Mathematical Statistics and Applied Mathematics Section has seriously reduced the capability of providing consultation from this group. While many opportunities exist for additional studies and additional training in cancer epidemiology and biometry, restrictions on available positions and other resources will force the programs to continue at essentially their present magnitude and scope. Because of the importance of these areas and the need to train additional people, it is urged that special attention be given to this area in the training programs of NCI, perhaps along the lines of special emphasis given to the problem in radiotherapy during the past few years.

Additional details and other information are given in the reports of the Epidemiology and Biometry Branches and the Epidemiologic Pathology Unit.

Summary Report
EPIDEMIOLOGIC PATHOLOGY UNIT
July 1, 1967 - June 30, 1968

> "Cancer research is of two main types: studies in causation and prevention and studies in diagnosis and treatment. Many different disciplines are concerned, but central to both forms of research and forming a bridge between them are pathology and epidemiology, which define the nature and extent of the problems to be solved." (1)

The first requirement for definition is a vocabulary for cancer that has pathological, epidemiological, clinical, and biological meaning. We are continuing our efforts to expedite development of a common national and international nomenclature, to bring the experts on animal cancer into a closer communication with those dealing with cancer of humans, and to get significant, behavioral definitions for the individual cancer species recognized by the pathologist. These behavioral definitions merge into specific studies of particular epidemiologic and clinical problems that require the generation and utilization of quantitative pathological data. Such efforts are described in the reports of the Migrant Studies of the Biometry Branch as well as in our own studies of multiple cancer. The third step which we are just beginning to plan for will be to move from the analysis of manifest cancer into the key region of pathogenesis. Here is where we would seek understanding of the beginnings of the various kinds of cancer and of the ways the developmental processes might be aborted. The places to study pathogenesis of course are the site of epidemics and the achievements of pathology in this area will continue to depend in good part on the extent to which we can utilize foreign as well as internal "experiments of nature" in our search for the causes of cancer in Americans

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(1) Editorial, British Medical Journal 1966, 2, 5

Serial No. NCI 4399
1. Epidemiologic Pathology Unit,
OASDD, ET
2. Office of the Head
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Incidence Studies of Multiple Primary Cancers

Previous Serial Number: Same

Principal Investigator: John W. Berg

Other Investigators (Not NCI): Robert V. P. Hutter*, David Schottenfeld*, Frank Ritter*

Cooperating Institution: *Memorial Hospital for Cancer and Allied Diseases, New York City

Man Years:

Total:	.8
Professional:	.3
Other:	.5

Project Description

Objectives:

To analyze rigorously the true incidence of subsequently developing primary cancers in populations of cancer patients and thereby identify high risk situations and epidemiologically and etiologically important patients subsets.

Methods Employed:

The man years of observation for patients divided by age and sex are calculated for the entire Memorial Registry population of 50,000 using the computer facility of the Department of Medical Physics, Memorial Hospital. The charts of patients with more than one cancer are then studied in detail. Pathologic material from the cases is reviewed to clarify controversial situations. Detailed investigations of high risk situations are to be undertaken by David Schottenfeld.

Major Findings:

In the study as a whole 793 cancers were expected in organ systems other than that of the first cancer. Twice as many, 1643, were found. Part of the increase seems to be procedural due to earlier detection but most is due to specific associations. Likely associations are now receiving a third level review to establish their import. One report has already been accepted for publication on a very high unexpected incidence of breast cancer following salivary gland cancer.

Significance of the Program to the Institute

The Memorial case file with its high concentration and specific follow-up represents the best material in existence for this type of study. The material is readily accessible, and particularly adequate for pathological review. It can be studied in a way fully compatible with similar ongoing Connecticut studies. Thus confirmation is available for results obtained for the more prevalent cancers while for rarer types the sets are directly additive giving answers not available from either source alone.

Proposed Course of Project:

The survey and related calculations were completed in fiscal '68. Studies of high risk situations should be completed within nine months. It is then planned that equivocal problems will then be studied in Connecticut.

Publications

Berg, J. W.: The incidence of multiple primary cancers. I. Development of further cancers in patients with lymphomas, leukemias and myeloma. J. Nat. Cancer Inst. 38: 741-752, May 1967.

Berg, J. W., Hutter, Robert V. P., and Foote, Frank W.: The unique association between salivary gland cancer and breast cancer. JAMA (in press).

Serial No. NCI 4398
1. Epidemiologic Pathology Unit,
   OASDD, ET
2. Office of the Head
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Registry Based Pathology Studies of Cancer

Previous Serial Number: Same

Principal Investigator: John W. Berg

Other Investigators (Not NCI): Robert V. P. Hutter***, Glenn Leak*, Robert Lukes**, Henry Eisenberg****, Weldon Bullock**, Constance Percy*****

Cooperating Units: End Results Section, Biometry; Departments of Surgery, Pathology and Cancer Registry, Memorial Hospital for Cancer and Allied Diseases, New York City***; Buffalo General Hospital, Buffalo, New York*; Pathology Department, Los Angeles County Hospital, Los Angeles, California**; Connecticut State Cancer Registry****; American Cancer Society, New York City*****.

Man Years:

Total:	2.0
Professional:	0.5
Other:	1.5

Project Description

Objectives:

1. To analyze pathology data now existing in tumor registries by frequency studies and correlations with other information on the patients.

2. To utilize existing registry data in the solution of specific pathology problems such as the significance of specific histologic patterns and the behavior of rare tumors.

3. To discover what further pathology data are needed and could be used in epidemiological and end-result studies and which types of Registry data are particularly needed to help solve problems in epidemiological pathology.

4. To plan for and collect such data in a form suitable for modern types of computer-based statistical analysis.

Methods Employed:

Computer surveys of large record files, record review of specific sets of cases, and microscopic studies of particular pathology material all are used when appropriate.

Major Findings:

1. Studies on nomenclature and classification of tumors have been expanded. The original work with pathologists (in the Systematized Nomenclature of Pathology of the College of American Pathologists) continues. With the assistance of this Unit and support from the Biometry Branch Contract with Tohoku University (64-10), the Japanese Pathological Society has prepared a Japanese version of this code. This achievement in turn has increased the interest of CIOMS in sponsoring similar equivalents in European languages and technical discussions are underway. The cancer section has been incorporated in the American Cancer Society's proposed new Tumor Nomenclature. This is scheduled for summer (1968) publication. As part of the testing program we arranged for the Cancer Society to support the coding and analysis of the complete special case Registry maintained by Dr. Bullock for the California State Society of Pathologists. The tabulation has proved an invaluable reference for our own work, particularly our consultations for the ICDA*. Publication is expected in the coming year.

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* Eighth Revision International Classification of Diseases, Adopted for Use in the United States. 1968.

2. A comprehensive study of the histologic contents of the Memorial Hospital Cancer Registry has been carried out and is now being prepared for monograph publication. A parallel study that will cover incidence as well as presentations and end results has been contracted for in Connecticut. Together these surveys should provide uniquely valuable reference points for future epidemiologic and end-result studies.

3. The original studies on esophageal cancer indicated that there were major ethnic and/or religious differences related to differences in the pathology of disease in Connecticut. These relations as well as the relation of sex to survival are being explored in much greater depth in several series of cases from hospitals of the End ResultsGroup.

4. Our Breast cancer data from Buffalo strongly suggests that 20-year survival is directly related to the completeness of radical mastectomy. The problems of analysis, while largely overcome has prompted exploration of more precise techniques for incorporating pathological measurements into clinical analyses. (See also (1)).

5. The prevalence of colon cancer was determined in 20,000 autopsies from Los Angeles County Hospital and is being analyzed by age and race as baseline for ongoing and proposed international studies of the Biometry Branch.

6. The possibility of using kidneys from patients with certain kinds of cancers for transplantation was investigated but found to be unsafe. (2).

Significance of the Program to the Institute·

While individual studies should have intrinsic value, the prime aim of the project is to provide a broad factual framework for use in planning the programs of the Etiology Area. In particular it aims for a more specific identification of the heterogenous assortment of diseases included in "cancer" of the various anatomic sites.

Proposed Course of Project:

As studies now underway are completed, others will be added in the light of the concerns of the Etiology Area and the Unit's capacity. We also hope to increase our efforts to disseminate the accumulated knowledge within and without this Area through our growing involvement with other groups dealing with the pathology, nomenclature and staging of cancer. The way has been opened for major steps at the international level but the present limitation on foreign travel of course will limit significantly such activity, even though a fully internationalized system of registration of tumor pathology remained our goal.

Honors and Awards

Berg, J. W.: Invited session chairman, UICC Symposium on Cancer Registries, Madrid, May 1968; Member, Task Forces on Lung Cancer, Melanoma and Breast Cancer, American Joint Committee on Cancer Staging and End Result Reporting; Member, Committee on Nomenclature and Classification of Disease, College of American Pathologists; Member, Task Force to Revise "Manual of Tumor Nomenclature," American Cancer Society; Member, Committee on Study of Migrant Populations, UICC; Consultant in Epidemiologic Pathology, Memorial Hospital for Cancer and Allied Diseases.

Publications

Berg, J. W., and Robbins, G. F.: The failure of a model to predict cancer survival. J chron. Dis. 1967, Vol. 20, pp. 809-814.

Wynder, Ernest L., and Berg, J. W.: Cancers of the lung among nonsmokers. Special reference to histologic patterns. Cancer 20: 1161-1172, (July) 1967.

Muiznieks, Helga W., Berg, J. W., Lawrence, Walter, Jr., and Randall, Henry T.: Suitability of donor kidneys from patients with cancer. Submitted for publication.

Serial No. NCI 4397
1. Epidemiologic Pathology Unit,
OASDD, ET
2. Office of the Head
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Comparative Pathology of Leukemias and Lymphomas of Animals and Man

Previous Serial Number: Same

Principal Investigator: Carolyn H. Lingeman

Other Investigators: None

Cooperating Units: Epidemiology Branch, National Cancer Institute, Bethesda, Maryland; Veterinary Pathology Section, Armed Forces Institute of Pathology, Washington, D.C.; Pathology Panel of Lymphoma Task Force of the NCI; WHO International Reference Center for Comparative Oncology

Man Years:

Total:	1.0
Professional:	1.0

Project Description

Objectives:

1. To compare morphologic characteristics of leukemias, lymphomas, and related diseases of vertebrate animals with the corresponding diseases of man as a baseline for epidemiologic surveys.

2. To attempt to relate differences in incidences and clinical and pathologic characteristics of these diseases in different species of animals to inherent characteristics of these animals and to environmental influences.

Serial No. NCI 4397

Significance of the Program to the Institute:

Pathologists must provide support for epidemiologic and experimental investigations of hemic neoplasms by providing a sound morphologic basis in terms which are consistent and applicable to a wide range of species of animals. Because neoplasms of this group have been best characterized in man, the nomenclature and system of classification which has been developed by medical hematopathologists is an appropriate model by which hemic neoplasms of other species may be compared. Comparative studies of hemic neoplasms and related immunoproliferative disorders may identify additional animal models for experimental studies.

Proposed Course of Project:

1. To continue to investigate comparative aspects of hemic neoplasms of animals by collection and examination of histologic and hematologic material. These will be evaluated in terms of the morphologic criteria which have been developed for describing these diseases of man.

2. To assist in preparation and distribution of study sets containing histologic and hematologic material from animals and man with characteristic examples of these neoplasms.

3. To encourage veterinary pathologists to adopt the terminology developed for these neoplasms of man.

4. To develop sources of material from veterinarians, in which there is adequate clinical, including hematologic, and complete necropsies. These sources will include veterinary pathology departments of universities, zoos and practicing veterinarians.

Methods Employed:

1. In cooperation with veterinary pathologists of the newly developed Comparative Pathology Branch of the Armed Forces Institute of Pathology, systematic morphologic study of histologic and hematologic material from animals with leukemias and lymphomas is being undertaken, with emphasis on subhuman primates, cats, dogs, cattle and swine. A special search is being made for examples of these diseases in subhuman primates because knowledge is limited to only 21 reports. Classification of these neoplasms are being based on those which have been developed for defining corresponding diseases of man.

2. A symposium "Comparative Morphology of Hematopoietic Neoplasms," jointly sponsored by the Epidemiologic Pathology Unit, NCI and the Veterinary Pathology Section, AFIP, was held at the AFIP March 11-12, 1968. Participants were veterinary and medical pathologists with specific knowledge and experience with hemic neoplasms of specific groups of animals. The systematic review included hemic neoplasms of invertebrate and lower vertebrate animals, avians, and mammals, including man. The members of the Pathology Panel of the Lymphoma Task Force provided the morphologic definition of hemic neoplasms of man. Proceedings will be published as a monograph.

Major Findings:

Almost all spontaneous hemic neoplasms of the mammals which have been studied are of lymphoid origin; myeloid forms of leukemia are rare. The spectrum of histologic types of lymphomas, a majority of which are non-leukemic, are similar to those which have been observed in man.

There is little knowledge concerning prevalence and morphologic types of hemic neoplasms of subhuman primates; to date, there have been only 21 reports, a majority of which do not include sufficient details for evaluation. Review and study of this particular group of tumors hence seems particularly important.

Summary Report
BIOMETRY BRANCH
July 1, 1967 - June 30, 1968

> "Not everything that is counted counts. Not everything
> that counts can be counted"
>
> S. Ross

The careful unbiased collection of data to be translated into a basis for action for the prevention, diagnosis, or treatment of cancer has characterized the activities of the Branch for the past year. If present plans are followed it will characterize the Branch activities for the next several years.

Two major data-gathering programs were "site-visited" during the year, the Norwegian-British migrant studies, and the End Results program. The site visitors were uniformly favorably impressed. Their major recommendations were for extensions and expansions. Our limited resources -- personnel and finances -- make it unlikely that these changes could be introduced in the immediate future. The third major data collecting program of the Branch, the Third National Cancer Survey has had preliminary review by the Bureau of the Budget and all aspects of it, including the gathering of socio-economic data, have been approved for the pre-test study in Birmingham, Alabama.

Consulting activities of the Branch, in Mathematics, Experiment Design, Systems Analysis (and related computer activities) have strained the available resources. With two senior mathematical statisticians leaving the Branch, the consulting burden has fallen increasingly on the younger staff. In the computer-related areas a good professional staff is being assembled, but demands for consultation have been so great that it has been impossible to extend regular consultation outside the Branch. However, a survey was made of the Veterinary Medicine Data program (Epidemiology Branch), and the first steps taken in the study of the computer needs of the entire Etiology area.

The main thrust of the Branch's data analysis has been in the direction of defining the individual cancer patient -- or potential patients. The migrant study programs are directed toward finding personal characteristics or exposures associated with the development of cancer -- or with its prevention. The first important publications from this study should be completed within a year or 18 months. The End Results group has turned its attention toward prognostic factors in specific diseases, in an attempt to find what in the patient, or his disease contributes to survival. It is also the aim of this program to uncover the patient characteristics that indicate one form of therapy in preference to another. These results are very much sharpened by adding the knowledge of the Epidemiologic Pathology Unit. A second generation cooperative clinical trial in cancer of the prostate with the Veterans Administration is directed toward defining patient characteristics that will lead to optimal treatment at optimal times in the course of the disease. Techniques derived from a simplistic model of breast cancer (developed by the End Results and Mathematical Statistics Sections as reported last year, and programmed for computation by Demography Section personnel) have been employed in forecasting survival of lung cancer patients in a controlled clinical trial. This model applied to this trial is helping develop a strong indication of which patients have a high probability of benefiting from chemotherapy and which do not. The work on early diagnosis through mammography continues. The first reports are encouraging -- and the patient population in which mammography appears to be a worthwhile control measure is becoming more clearly defined.

The last annual report reported the development (and modification over time) of a model for breast cancer which showed promise for indicating choice of treatments. Further developments and changes are generalizing this model to possible implications for prevention as well as treatment.

Study of a large population of ministers has shown them to be at a much lower risk of death (especially from lung cancer) than the general population of men of the same age and places of residence. No simple explanations are immediately at hand. This work will need a detailed follow-up.

The End Results group has completed the third major review of incidence, mortality and survival from cancer in "End Results in Cancer, Report #3 (to be distributed in September 1968 at the National Cancer Conference). Several important trends are worth noting.:

1. There has been a substantial increase in cancer of the colon -- balanced by improved survival of the patients.

2. The stable mortality in breast cancer over the last several decades is a result of a substantial increase in incidence in younger women -- balanced by increased survival.

3. There is evidence that the increases in survival achieved by specialty institutions and research programs in acute lymphocytic leukemia are also being experienced by the "general run" institutions, tho not in so dramatic a fashion.

4. Increases in survival occurred in many forms of the disease during the 1950 decade when compared to the 1940 decade. The gains recorded in the 1960 decade are not as large as those achieved earlier.

5. Lung cancer incidence continues to increase -- but survival, particularly of the localized form of the disease, has improved substantially.

Recognizing that much of the medical profession is unaware of these trends, The End Results group is now preparing a summary of Report Number 3 to appear in one issue of the general cancer magazine Ca. In addition several reports of a non-technical nature are being prepared for publication in a range of general medical journals.

A substantial portion of the past year has been devoted to the planning of the 3rd National Cancer Survey with extensive support by the ADP management staff. An information brochure for physicians is attached. The general study will be preceded by a three month pre-test in Birmingham, Alabama. A contract has been let for the systems analysis and data processing for the pre-test. The final version of the questionnaires and procedures will be determined by the pre-test experience, which will also provide a basis for close estimation of costs for the entire survey. A Special Cancer Survey Section has been established to carry out the pre-test and the full survey. All public relations activities with hospitals and physicians participating in the survey are closely coordinated with and are receiving support from the field organizations of the American Cancer Society.

The mathematical and statistical consulting activities of the Branch have extended into two new areas -- problems of immunology, and problems of pattern recognition by automated devices. A two-stage (but not two-disease) mathematical model of breast cancer is being investigated in collaboration with Dr. Berg of the Epidemiologic Pathology Unit. The adequate development of this model will involve at least one full-time mathematical-statistical consultant. Extensive consultation has been given on problems of disease (and other) clustering, and carcinogenesis. Technical developments in the field include the development of tests of the folded binomial distribution, linear models of cell biochemistry, a treatment vs. control median test, a measure of the power of the modified Wilcoxon test, extension of a mathematical theory of epidemics, small sample tests for the Poisson distribution, a model of shock, some developments in genetic theory, new clinical trials designs, models of bacteriophage growth, ranking procedures for arbitrarily restricted observations, the use of positive and negative controls, some developments on U-statistics and their relationship to the "jackknife" method, simultaneous confidence interval estimation, restricted least squares regression, and computation of indirect adjusted rates in the presence of confounding.

Consultation with the Regional Medical Programs has fallen off. The RMP group apparently now feel able to convey our experience to the Regional groups who will be developing disease registers, and data gathering and reporting systems. Consultation with other agencies (e.g. Food and Drug Administration as the prime recipient) has continued at about the same level as in the past.

Administrative notes and plans

A review has begun of data collected in cooperation with the Railroad Retirement Board to assess the risk of different railroad occupation groups. If review indicates that something fruitful can be gained by detailed pursuit of this study, personnel will be assigned to complete this work.

The major needs of the Branch lie in the consulting areas. Our Mathematical Statistics staff has had difficulty in recruitment other than by use of commissioned officer appointments. This has brought us promising young people -- whom we hope will remain with us as they mature. Some positions will be added to the Data Processing Management Staff, but not yet nearly enough to meet the needs of the Etiology area -- thus restricting our consultation services sharply. This group shows promise of making an important contribution to the Cancer Institute Program and we contemplate constituting it as an independent unit within the Branch.

Although site visit teams have recommended extensions and expansion of the migrant studies and the End Results Program, unavailability of positions precludes any expansion. The major new data-gathering activities in the coming year and in several years to come will center about the 3rd National Cancer Survey, and the development of a detailed leukemia registry - the latter oriented toward developing patient prognostic characteristics.

Honors and awards received by Branch staff members in the past year include:

John C. Bailar, III

a) Invited lecturer, Yale University (Biostatistics)
b) Invited paper, American Radium Society
c) Invited paper, New England Cancer Society
d) Invited participant, Conference on the Epidemiology and Etiology of Bladder Cancer
e) Contributed paper, American Statistical Association
f) Invited paper, American Urological Association (Mid-Atlantic Section)

Sidney J. Cutler

a) Invited participant in Symposium on Breast Cancer sponsored by the Canadian Cancer Society, March 8-9, 1968, Toronto, Canada.
b) Invited participant in Symposium on Cancer Registries, sponsored by International Union Against Cancer, May 2-4, 1968, Madrid, Spain.
c) Member, N.C.I. Breast Cancer Task Force.
d) Consultant, American Joint Cancer Staging and End Results Reporting.
e) Scientific Reviewer, Journal National Cancer Institute, Cancer, Cancer Research and Science.

John J. Gart

a) Reviewer, Biometrics, Journal of the American Statistical Association and Journal of Theoretical Biology.

b) Invited Lecturer, Johns Hopkins University.

William Haenszel

a) Invited participant in Symposium on Cancer Registries, sponsored by International Union Against Cancer, May 2-4, 1968, Madrid, Spain.
b) Member, U.I.C.C. Commission on Epidemiology and Cancer Prevention.
c) Chairman, U.I.C.C. Committee on Studies of Migrant Populations.
d) Chairman, Organizing Committee, International Association of Cancer Registries.
e) Adjunct Professor of Biostatistics, University of Pittsburgh Graduate School of Public Health.
f) Faculty member, Summer Session in Biostatistics at School of Medicine, University of Oslo, Oslo, Norway, June-August 1967.
g) Invited lecturer, Japan Public Health Association Annual Meeting, Sendai, Japan, October 1967.
h) Invited lecturer, Japan National Cancer Congress, Nagoya, Japan, October 1967.

Haitung King

a) Advisor-Consultant, D. C. Interagency Council on Smoking.
b) Clinical Associate Professor of Community Medicine and International Health, Georgetown University Medical School.
c) Invited to prepare core examination questions for the American Board of Preventive Medicine.
d) Review article on Epidemiology of the Urinary Bladder Cancer (with John C. Bailar, III) selected to be included in the Yearbook of Urology.
e) Invited paper (with John C. Bailar, III) presented at the annual meeting of Population Association of America.

Nathan Mantel

a) Consultant, Food and Drug Administration, Washington, D. C.
b) Member, Editorial Committee, Biometrics.
c) Nominated for Fellow, Royal Statistical Society
d) Member, Editorial Advisory Board, Cancer Research
e) Adjunct Professor of Biostatistics, University of Pittsburgh Graduate School of Public Health

Marvin A. Schneiderman

a) Invited lecturer, Radiological Society of North America -- Refresher Course in Statistics.
b) Invited lecturer, Walter Reed Institute of Medical Research
c) Invited papers, editor for Biometrics.
d) Regional Advisory Board Member, Biometric Society (ENAR)
e) Invited lecturer, Food and Drug Administration Scientific Associates Program.
f) Member, Biostatistics Fellowship Review Committee, NIH
g) Adjunct Professor of Biostatistics, University of Pittsburgh Graduate School of Public Health

Marvin A. Schneiderman (continued)

h) Clinical Associate Professor of Statistics, Georgetown University School of Medicine

Howard M. Shapiro

a) Presented paper at Sixth Annual Symposium on Biomathematics and Computer Science in the Life Sciences in Houston, Texas in March 1968

Donald G. Thomas

a) Presented paper at the National Meeting of Biometric Society in Washington, D. C. in December 1967.

Serial No. NCI-4250
1. Biometry Branch, OASDD, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Activities during FY 1968

Previous Serial Number: Same

Principal Investigators: William Haenszel, Marvin A. Schneiderman and Nathan Mantel

Other Investigators: Emily Dawson, Elias Kazam

Cooperating Units: None

Man Years:
Total: 9.0
Professional: 5.5
Other: 3.5

Project Description

1. General planning and direction of the scientific problem of the Branch, including recruitment and development of statistical staff and coordination of activities of statistical units assigned to other Branches of the National Cancer Institute. Mr. Haenszel serves as a member of the Scientific Directorate, NCI. Dr. Schneiderman is a member of the Cancer Chemotherapy contracts review committee.

Given prevailing parket conditions of supply and demand for biostatisticians and mathematical statisticians, the Biometry Branch's position with regard to senior staff members is as good as can be expected. Work on the cancer morbidity surveys to be done in the years around the 1970 census would be expedited by addition of two experienced statisticians to the staff, but it would be unrealistic to expect further staff expansion through recruitment of experienced senior statisticians. Future needs must be met by recruitment of junior staff who have just completed doctoral training or who have interrupted graduate training for work experience. We have been able to recruit some statisticians by offering commissions in Reserve Corps of the U.S. Public Health and we will continue to use this device.

The Biometry Branch staffing problems are becoming increasingly severe and our position with respect to capabilities for handling increased demands for consultant services is not good. In addition to providing consultation in experimental design and mathematical statistics we are confronted with the need for similar services in the area of systems design and computer programming. As a first step in this direction, Mr. Theodore Weiss was recruited during the past year and he will serve as the focal point in the Etiology Area for consultant services in systems and computer programming.

The trend to regionalization implicit in the Regional Medical programs may have a long-range impact on Branch programs. Regionalization will require and force great changes in systems for storing and retrieving medical data and these developments must be watched for their impact on existing activities within the Etiology Area. Of immediate concern is the effect on plans for the resurvey of cancer morbidity in the 10-city and the State of Iowa scheduled for the years around the 1970 population census. The selection of survey areas has been made with due note of regional center developments and we are prepared to integrate the morbidity surveys with plans for regional cancer registers as they arise and to participate actively in effecting a transition from the ad hoc morbidity surveys to cancer registers in selected areas.

The developments in regionalization also present an opportunity and a challenge to enlarge the scope and participation of institutions in the End Results Program now operated by the Biometry Branch. While the present network of reporting institutions yields an annual intake of 50,000 newly-diagnosed cancer cases, this number could readily be doubled, accompanied by a more balanced regional distribution of cases, by inclusion of some of the regional cancer centers. The need for better-balanced coverage of specialty and general hospitals in the several regions of the U.S. was recognized by the site visitors review of the End Results Program in December 1967.

2. Mr. Haenszel and Dr. Berg continued to work with Professor Correa of the Universidad del Valle on the cancer morbidity surveys for Cali, Colombia, and during the past year a research contract (PH43-66-907) was negotiated to support Correa's work in Colombia.

The correlation of the incidence data with pathology findings is the particular interest of Correa and his colleagues. Studies of autopsy materials and surgical specimens are underway with major attention to the definition of criteria whichdifferentiate between high- and low-risk populations as revealed by contrasts of morbidity data for cities within Colombia (Cali, Medellin, Cartegena) and by socio-economic class and place of birth.

In Cali development of an organ bank of tissues from persons dying from accidents and acute diseases, its feasibility having been demonstrated by a pilot trial, will be continued and utilized for studies of occult tumors and premalignant conditions. Some preliminary, but provocative, observations have been yielded by the medical examiner and hospital autopsy materials. Application of the Lauren concepts of intestinal and diffuse types of stomach cancer points to substantial differences between Cali where stomach cancer is common and in Cartagena where it is rare. Thyroid cancer in Colombia also reveals rather striking geographical differences by histologic type and studies in Colombia and selected other countries promise to yield leads useful for work on thyroid cancer in the U.S.

3. The Biometry Branch continued to provide consultation for the research contract with the Health Insurance Plan of Greater New York to evaluate mammography as a screening tool for the control of breast cancer, for which Mr. Haenszel and Dr. Cutler are the project officers; the study protocol was drafted in great detail by the Branch and requires monitoring the project to see that work proceeds according to plan.

The full scale field trial has been underway for four years. The findings to date are encouraging and consistent with the hypothesis that mammography may contribute significantly to detection and lead to earlier detection of breast cancer. Further surveillance of the study populations will be carried out in line with original plans, although detailed modifications will be introduced as the study unfolds. Clearly, the longitudinal study of 60,000 women has potential for related epidemiological investigations of breast cancer and proposals to take advantage of this opportunity will be developed in consultation with the NCI Breast Cancer Task Force. During the past year 40 cc of blood was collected from the women subjected to mammography and stored for later use.

The most recent development regarding utilization of the stored, frozen blood plasma is the pilot study by Drs. Griff Ross and Mortimer Lipsett of the Endocrinology Branch of the relationship of gonadotropin levels in blood plasma to time elapsed after menopause. Initially, determinations will be run on a limited number of samples to provide information on possible trends and on variability of gonadotropin levels at various postmenopausal intervals. Ultimately, they hope to investigate the relationship of blood plasma gonadotropin levels to the risk of developing cancer of the breast and to prognosis.

4. Mr. Haenszel serves as a project officer for a Public Law 480 agreement with the Indian Cancer Society for support of a cancer morbidity survey in Bombay, India. Negotiations for a 3-year extension of work from 1968 to 1971 have been completed.

A publication entitled "Cancer in Greater Bombay" reports on cancer incidence, based on residents of the area first diagnosed as having cancer in the calendar year 1964. Deaths previously unreported as cases account for approximately 25% of the newly-diagnosed cases in 1964; of the new cases ascertained by review of hospital and physicians' records, approximately 65% are confirmed by histological examination. A paper updating the results of the morbidity survey through 1966 has been submitted to the British Journal of Cancer. The Bombay study will yield epidemiological data on cancer of the oral cavity (a high risk site for the Hindu population) and will also provide a systematic descriptive data for Parsis who present a quite different pattern of cancer risks, notably high rates for lung and breast, from those in the Hindu population.

The original plans called for extension of the Bombay survey in 1968 to cover selected areas in Gujrat and Maharashtra states. Work to date indicates that continued concentration on and intensive exploitation of the Bombay study situation would be more rewarding and the time table for extension to other areas in Maharashtra state has been postponed to 1970 or 1971. We anticipate that Bombay can be used as a training center for persons recruited to carry out similar surveys elsewhere in India. The use of the Bombay group as a nucleus for development of surveys and register-based investigations elsewhere in India is greatly facilitated by cooperation with the Demographic and Research Training Center located nearby.

5. Mr. Haenszel serves as a project officer for a Public Law 480 agreement with the Oncological Institute (Warsaw) to support development of a comprehensive cancer case reporting scheme in selected urban and rural areas of Poland. Work in Poland will provide baseline observations required for studies of shifts in cancer risks among Polish migrants to the United States. Cancer registers have been established and are now functioning in the Warsaw, Minsk, Siedlice districts and two districts near Gliwice (total population, 1,800,000), in Novy Sacz county and Krakow city (total population, 600,000). Registration was introduced during the last year in the districts of Cieszyn and Swietochłowice (total population, 200,000). The program directed by Professor Koszarowski and Dr. Staszewski over the past 4 years has been highly successful. The program will be supported for an additional 2 years through 1969 with PL-480 funds. This project (and related ones) received very favorable comment in a British Medical Journal editorial.

6. Mr. Haenszel as chairman of the Committee on Study of Migrant Populations of the International Union Against Cancer has been active in planning a work shop on the methodological aspects of studies of migrant populations to be held in Honolulu during February 1969. The activities of this Committee provide a useful complement to the present Branch activities in studies of shifts in cancer risks among migrant populations, which are directed to foreign-born groups in the United States.

7. Mr. Mantel and Dr. Schneiderman have provided consultation to the Food and Drug Administration on problems of experiment design, safety testing, establishment of the "accuracy of a method", optimal dose finding, combination drug testing and several related areas. Dr. Schneiderman was extensively involved in consultation related largely to clinical research, including the design of several clinical trials, clinical laboratory techniques, search for virus particles in cancer patients, and the completion of several cooperative clinical trials with the Eastern Cooperative Group.

8. Mr. Mantel has been conducting an extensive program of statistical research and consulting. This work has involved other statisticians within the Branch, other research workers outside the Branch, including investigators within the Etiology Area of the National Cancer Institute, elsewhere within the NCI and outside the NCI. He has been the statistical advisor on various projects related to the work of the National Cancer Institute. These consulting activities have motivated much of Mr. Mantel's statistical research work, including development of statistical procedures and statistical theory. In calendar year 1967, this was reflected in the appearance of a total of 18 publications for which Mr. Mantel was either an author or coauthor.

A description and partial listing of some of these activities follow:

a) Consulting within the Etiology Area, NCI
   i. Birth order, maternal age, temporal, spatial and seasonal clustering in leukemia and mongolism (Stark)

   ii. Canine neoplasms.(Tjalma, Priester)

   iii. Viruses in cancer and leukemia.(Miller)

   iv. Miscellaneous epidemiological and demographic studies. (Fraumeni, Oleinick, Glass, Miller, Bailar, Haenszel, List, Berg, Kazam, Lee)

   v. Carcinogen testing. (Weisburger)

   vi. Evaluation of survival data. (Cutler, Bailar, Scotto)

   vii. Miscellaneous biological problems. (Bates, DiPaolo, Peacock, Chirigos, Rapp, Borsos)

b) Consulting outside the Etiology Area, NCI
   i. Screening and testing of cancer chemotherapeutic agents. (Goldin, Schepartz, etc. CCNSC)

   ii. Epidemiological studies and studies of disease clustering. (Mustacchi, Klauber, University of California Medical Center; Pike, Medical Research Council, and various others).

   iii. Air pollution studies. (Epstein, Children's Cancer Research Foundation).

   iv. Endocrine studies.(Vollmer, Endocrine Evaluation Branch, NCI).

   v. Studies of toxicity and carcinogenicity (Epstein, Children's Cancer Research Foundation).

   vi. Drug safety and efficacy and related problems. (Gross, Winbush and others, FDA)

vii. Studies of combined drug actions. (Goldin, CCNSC and miscellaneous others)

viii. Statistical methods in genetics. (Li, University of Pittsburgh)

ix. Statistical methodology. (Greenhouse, NICHD; Pasternack, N.Y.U. Medical Center)

c) Statistical research and publications.

During the past year 6 papers on statistical methodology by Mantel have been published, with 3 others in press. Additionally 9 subject matter papers frequently reflecting this methodology, with one including a methodological appendix, have been published, with 4 others in press. The methodological papers cover a wide range of subjects including: evaluation of data subject to restricted observation; maximum likelihood and moments in probit analysis; use of positive controls; disease clustering; U statistics and the jackknife method; Kärber's method for the exponential parameter; estimation of the segregation ratio; simultaneous confidence intervals; occupancy problems; statistical methods in carcinogenesis studies.

Mr. Mantel has served as a formal referee for approximately 30 scientific papers in the past year, these representing submissions to a wide range of professional journals. He has also provided informal reviewing for many manuscripts submitted directly to him by the authors. Additionally he has formally reviewed 2 statistical texts, these reviews to appear in the coming year.

Honors and Awards

Haenszel, W.: Invited participant in Symposium on Cancer Registries, sponsored by International Union Against Cancer, May 2-4, 1968, Madrid, Spain. Member, UICC Commission on Epidemiology and Cancer Prevention; Chairman, UICC Committee on Studies of Migrant Populations; Chairman, Organizing Committee, International Association of Cancer Registries; Adjunct Professor of Biostatistics, University of Pittsburgh Graduate School of Public Health; Faculty member, Summer Session in Biostatistics at School of Medicine, University of Oslo, June-August 1967; Invited lecturer, Japan Public Health Association Annual Meeting, Sendai, Japan, October 1967; Invited lecturer, Japan National Cancer Congress, Nagoya, Japan, October, 1967.

Schneiderman, M.A.: Invited lecturer, Radiological Society of North America--Refresher Course in Statistics; Invited lecturer, Walter Reed Institute of Medical Research; Invited papers editor for Biometrics; Regional Advisory Board member, Biometric Society (ENAR); Invited lecturer, Food and Drug Administration Scientific Associates Program; Member, Biostatistics Fellowship Review Committee, NIH; Adjunct Professor of Biostatistics, University of Pittsburgh Graduate School of Public Health; Clinical Associate Professor of Statistics, Georgetown University School of Medicine.

Mantel, N.: Consultant, Food and Drug Administration, Washington, D.C. Member, Editorial Committee, Biometrics; Member, Editorial Advisory Board, Cancer Research; Nominated for Fellow, Royal Statistical Society; Adjunct Professor of Biostatistics, University of Pittsburgh.

Publications

Haenszel, W.: Epidemiology of gastric cancer. In Neoplasm of the Stomach (McNeer, G. and Pack, G.T.). Philadelphia, J.B. Lippincott Company, pp.3-28, 1967.

Haenszel, W.: Concept, measurement, and data in migration analysis. Demoraphy IV: pp. 253-261, 1967.

Haenszel, W. and Segi, M.: Stomach Cancer Among The Japanese. In Harris, R.J.C. (Ed): Proceedings Ninth International Cancer Congress, Tokyo, October, 1966. U.I.C.C. Monogr. Series, Volume 10, pp. 55-63, Heidelberg, Springer-Verlag, 1967.

Haenszel, W. and Kurihara, M.: Studies of Japanese migrants. I. Mortality from cancer and other diseases among Japanese in the United States. J. Nat. Cancer Inst. 40, 43-68, 1968.

Taeuber, K., Chiazze, L. Jr., and Haenszel, W.: Migration in the United States: An analysis of residence histories. Public Health Rep. Monogr. (in press).

Carbone, P.P., Spurr, C., Schneiderman, M., Scotto, J., Holland, J.F. Shnider, B.: Management of patients with malignant lymphoma: A comparative study with cyclophosphamide and vinca alkaloids. Cancer Res. 28, May 1968.

Schneiderman, M.A.: Quantitative thinking in medicine-biostatistics (using numbers to make the route from cause to effect and back). In Canter, P. (Ed): Traumatic Medicine and Surgery for the Attorney. London, Butterworth & Co. (in press).

Gold, G. L., Shnider, B. I., Salvin, L. and Schneiderman, M.A.: The use of mechlorethamine, cyclophosphamide, and uracil mustard in neoplastic disease: A controlled study. (In press)

Epstein, S.S., Joshi, S., Andrea, J., Clapp, P., Falk, H., and Mantell, N.: Synergistic toxicity and carcinogenicity of "freons" and piperonyl butoxide. Nature 214: 526-528, April 1967.

Mantel, N.: Ranking procedures for arbitrarily restricted observations. Biometrics 23: 65-78, March 1967.

Mantel, N.: Leukemia clustering in the Liverpool area? Lancet: 1058-1059, May, 1967.

Mantel, N. and Greenhouse, S. W.: Equivalence of maximum likelihood and the method of moments in probit analysis. Biometrics 23: 154-157, March, 1967.

Mantel, N. and Stark, C. R.: Paternal age in Down's syndrome. Amer. J. Ment. Defic. 71: 1025, May 1967.

Gross, A. and Mantel, N.: The effective use of both positive and negative controls in screening experiments. Biometrics 23: 285-295, June 1967.

Stark, C. R. and Mantel, N.: Lack of seasonal- or temporal-spatial clustering of Down's syndrome births in Michigan. Amer. J. Epid. 86: 199-213, July, 1967.

Baer, P. N., Hawkins, G. R., Wells, H., Mantel, N., and Zipkin, I.: Studies on experimental calculus formation in the rat. XI. Relation to diet and selected salivary constituents. J. Periodont. 38: 323-329, 1967.

Epstein, S. S., Andrea, J., Joshi, S., and Mantel, N.: Hepatocarcinogenicity of griseofulvin following parenteral administration to infant mice. Cancer Res. 27, 1900-1906, 1967.

Epstein, S. S., Andrea, J., Jaffe, H., Joshi, S., Falk, H., and Mantel, N.: Carcinogenicity of the herbicide maleic hydrazide. Nature 215: 1388-1390, 1967.

Mantel, N.: Assumption-free estimators using U statistics and a relationship to the jackknife method. Biometrics 23: 567-571, 1967.

Stark, C. R. and Mantel, N.: Temporal-spatial distribution of birth dates for Michigan children with leukemia. Cancer Res. 27: 1749-1755, 1967.

Mantel, N.: Adaptation of Karber's method for estimating the exponential parameter from quantal data, and its relationship to birth, death and branching processes. Biometrics 23: 739-746, 1967.

Li, C. C. and Mantel, N.: A simple method of estimating the segregation ratio under complete ascertainment. Amer. J. Hum.Genet. 20: 61-81, Jan. 1968.

Epstein S. S., Andrea, J., Clapp, P., Mackintosh, D., and Mantel, N.: Enhancement by piperonyl butoxide of acute toxicity due to freon, benzo(a)pyrene and griseofulvin in infant mice. Toxic. Appl. Pharmacol. 11: 442-448, 1967.

Mantel, N.: Simultaneous confidence intervals and experimental design with normal correlation. Biometrics (in press).

Mantel, N. and Pasternack, B. S.: A class of occupancy problems. Amer. Stat. (in press).

Mantel, N.: Some statistical viewpoints in the study of carcinogenesis. Progr. Experi. Tumor Res. Vol. 11 (in press).

Epstein, S. S., Mantel, N., Stanley, T. W.: Photodynamic assay of neutral subfractions of organic extracts of particulate atmospheric pollutants. Environ. Sci. Technol. 2: 132-138, 1968.

Goldin, A., Venditti, J. M., Mantel, N.: Evaluation of combination chemotherapy with three drugs. Cancer Res. (in press).

Epstein, S. S. and Mantel, N.: Hepatocarcinogenicity of the herbicide maleic hydrazide following parenteral administration to infant Swiss mice. Arch. Environ. Health. (in press).

Kline, I., Mantel, N., Gang, M., Tyrer, D. D., Venditti, J. M. and Goldin, A.: Duration of drug levels in mice as indicated by residual antileukemia efficacy. Chemotherapy. (in press).

Serial No. NCI-4262
1. Biometry Branch, OASDD, Etiology
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Statistical Consultation and Services for the Eastern Cooperative Oncology Group

Previous Serial Number: Same

Principal Investigator: Marvin A. Schneiderman

Other Investigators: John J. Gart, Joseph Scotto

Cooperating Units: Eastern Cooperative Oncology Group, Operations Office, 850 Sligo Avenue, Silver Spring, Md.; Albany Medical College of Union University, Albany, N.Y.; Boston USPHS Hospital, Boston, Mass.; Georgetown University Medical School Chemotherapy Program; Harvard Cancer Commission, Walpole, Mass.; Jackson Memorial Hospital (University of Miami), Fla.; Johns Hopkins Hospital, Baltimore, Md.; Lemuel Shattuck Hospital, Boston, Mass.; Maimonides Hospital, Brooklyn, N.Y.; Roswell Park Memorial Institute, Buffalo, N.Y.; National Cancer Institute, National Institutes of Health, Bethesda, Md.

Man Years:

Total:	3.0
Professional:	1.0
Other:	2.0

Project Description

Provision of consultative and clerical services required for the collection, tabulation, analysis, interpretation and presentation of data arising out of clinical trials of drugs as conducted by the Eastern Cooperative Oncology Group.

Objectives:

The primary objective is to provide a sound statistical foundation for the clinical trials conducted by this Group, and to assist in the intelligent evaluation of the data derived from the trials.

Methods Employed:

Experiment design planning from the point of view of modern theory of inference, review of protocols, assistance in preparation of reports, including all computer runs, life table computations, construction of mathematical models and estimation of parameters of these models--with the development of new techniques and the adaptations of old ones where necessary.

Major Findings:

Major medical findings are reported in the medical journals, in papers in which the Branch members are occasionally junior authors. Methodologic techniques and findings are also sometimes imbedded in these papers. In the past year these have been:

1. Patient characteristics at start of therapy are important prognostic factors. Clinical trials by the ECOG have shown that a patient's initial performance, hemoglobin count, and exposure to prior therapy, etc., determine to an appreciable degree the patient's subsequent behavior with respect to tumor response as well as overall survival. Indices of prognostic factors were devised which identified patients as "Poor", "Fair" or "Good" risks. The greatest differences of effective chemotherapy were observed for those patients in the "Fair" risk group. There is no general rule for identifying important prognostic factors. Each disease is unique, and a review of the history and nature of the disease is important before clinical trials begin so that inherent biases may be accounted for in the randomization scheme.

2. In diseases such as cancer where survival from date of diagnosis is relatively short, subjective measures such as a physician's prediction of a given patient's survival at outset of therapy may be a useful and practical measure when done in a conscientious manner. In one experiment it was found that patients expected to survive less than six months (poor risk) had a tumor response rate of about thirty percent, while patients expected to survive six to nine months (fair risk) had a rate of response of over fifty percent, and patients expected to survive nine months or more had a rate of response of over sixty percent. The physician's success at properly categorizing patients into relative risk groups and the association of initial status and survival suggests that the physician's keen observations and judgment in assessing patient-response although still considered a "subjective" evaluation should be scrutinized more carefully with efforts made to maximize their utilization in the analysis of clinical trials.

3. The designs of experiments for clinical trials do not generally provide for extensions of analyses beyond the scope of the original protocol. Much data accumulated at the close of a study are still valuable but severely limited in use. Our findings show that some investigators are not aware that analyses of data "after the fact" lack sufficient theoretical support.

Significance to the Program of the Institute:

The Eastern Cooperative Oncology Group is one of the oldest and most effective of the cooperative chemotherapy groups working in cooperation with the National Cancer Institute. The Group has pioneered in controlled clinical trials in cancer, in the development of measurement techniques and in the use of imaginative controlled-trial type of experiment designs in the earliest stages of drug evaluation. They have been particularly effective in:

1. Development of early dose-response schemes for rapid dose finding in man.

2. Screening programs for anti-tumor agents in man, including combinations of treatments.

3. Evaluation of therapeutic effect of specific anti-tumor agents.

4. Comparative trials of two or more anti-tumor agents to determine the relative efficacy of these agents in the treatment of human cancers.

5. Detecting differences in treatment and maintenance of patients between participating institutions which bear upon patients' eventual response to therapy.

Proposed Course of the Project:

This was the terminal year of this project. Future work will be conducted on a consulting basis as needed. Several papers are in preparation, or in press, and will be reported as they appear.

Publications

Schneiderman, M.A.: The Shin Tokaido to Developing New Treatments in Medicine: Unbiasedness. In Harris, R.J.C. (Ed.): Proceedings of the 9th International Cancer Congress, Tokyo, October 1966. U.I.C.C. Monogr. Series, Volume 10, pp. 194-198, Heidelberg, Springer Verlag, 1967.

Serial No. NCI-4262

Hall, T.C., Nevinny, H.B., Holland, J.F., Maldia, G., Schneiderman, M., Salvin, L., Shnider, B.I. (Chairman), Gold, G.L., Frei, Emil III, Carbone, P.P., Colsky, J., Franzino, A., Grob, D., Matiasevic, D., Olson, K.B., Horton, J., Owens, A., Santos, G.W., Chalmers, T.C., and Krant, M.J.: Comparison of antimetabolites in the treatment of breast and colon cancer. J.A.M.A. 200, No. 9: 770-778, May 1967.

Carbone, P.P., Spurr, C., Schneiderman, M., Scotto, J., Holland, J.F., Shnider, B.: Management of patients with malignant lymphoma: A comparative study with cyclophosphamide and vinca alkaloids. Cancer Res. 28, May 1968.

Serial No. NCI- 4253
1. Biometry Branch,
OASDD Etiology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Statistical Studies of Cancer in Pittsburgh Area

Previous Serial Number: Same

Principal Investigators: J. William Lloyd (DEHS), Frank Lundin (DEHS)

Other Investigators: None

Cooperating Units: University of Pittsburgh School of Public Health
Occupational Studies Unit (DEHS)

Man Years:
Total: 2.0
Professional: 1.5
Other: 0.5

Project Description

Objectives:

To pursue a series of intensive cancer morbidity and mortality studies in a single geographic area to obtain information on factors contributing to unusual incidence for specified sites. The collation of data obtained by a variety of approaches to the same population base will permit stronger inferences to be drawn from the chain of evidence, open the way to designing detailed studies to test specific hypotheses suggested by the evidence and make possible methodological contributions to field studies of chronic illness in human populations.

Methods Employed:

A cancer registry, which receives reports from nearly all hospitals in Allegheny County, is being operated in cooperation with the State and County health departments, and the School of Public Health. The information received from the hospitals was supplemented in 1959 by a canvass of private physicians' records pertaining to cancer patients seen in 1957 and 1958. The data obtained from private physicians has been collated with the hospital reports and listings of cancer deaths obtained from the Bureau of Vital Statistics. The collected material has been coded and entered on punch cards.

A second phase of the project has been opened via a contractual arrangement (Contract No. PH43-62-147) with the School of Public Health for which Drs. Frank Lundin and J. William Lloyd are Project Officers. This second phase involves study of the mortality experience of a cohort of 59,000 men employed in the Allegheny County steel industry in 1953. These men are being followed through December 31, 1966. Occupational histories and pertinent demographic information have been abstracted from employment records and the vital status of each employee has been determined by follow-up studies. Coke-oven workers, who were shown in this study to have very high lung cancer rates, are being studied in several locales outside the Pittsburgh area to strengthen the data especially for the Caucasian race. Mr. H. W. Rush is now stationed in Pittsburgh and is in charge of the day-to-day activities of the field operations.

Major Findings:

1. Incidence rates for the Pittsburgh city portion of Allegheny County indicate that little change has taken place in the incidence of all forms of cancer since the previous survey in 1948. However, significant increases in the rate of male respiratory cancer and female breast cancer have been offset by substantial declines in the incidence of digestive cancer.

2. Significant intra-city variation of cancer incidence has permitted identification of areas of particularly high risk in 1957-1958.

3. There have been significant changes in the intra-city variation of cancer incidence between 1947 and 1957-1958. The nature of these changes will have a bearing on the methodological aspects of future studies of this type.

4. Nonwhite coke-oven workers employed at the "topside" of the ovens had lung cancer mortality rates markedly in excess of those predicted by the mortality experience of other nonwhite steelworkers. Mortality from other causes for this group was little different from that expected. No excess in mortality is apparent for white coke-oven workers. However, the number of white men employed "topside" is too small to rule out the possibility of excess lung cancer in this group. (Lloyd, J.W.: Lung Cancer Mortality in Allegheny County Coke Plant Workers, Doctoral dissertation, University of Pittsburgh 1966) NCI-4349.

Significance to the Program of the Institute:

The basic notion of epidemiological studies of a population in depth has never been attempted on this scale for cancer. These studies are contributing valuable information on methodology in field studies of chronic illness in human populations and the usefulness of population-based registers in a limited area. The nature of the intra-city variation indicates that the hypermobility of an urban population must be considered in an assessment of residence differences in the risk of disease. There is an indication of the approach future detailed studies must take to investigate the interaction of subsets of an urban population. The study of mortality among the steelworkers will provide information regarding the epidemiology of cancer as related to specific occupational exposures.

Proposed Course of the Project:

Tabulations have been secured for a comprehensive report of the incidence of cancer in 1957 and 1958 among all residents in Allegheny County of the type not available since the 10-cities studies of 1947 and 1948. Intra-community variations of cancer morbidity will be examined in the light of the steelworkers study to determine the extent to which differences in risk by residence may reflect occupational differences. In addition emphasis will be placed upon more specific definitions of the geographic, demographic and social variables related to cancer.

Additional follow-up of the present study populations as well as an additional group of white coke plant workers will be made to clarify the differential mortality pattern of the two races. Further analysis of mortality experience of other occupational areas in the steel industry will be carried out along the same lines as that for coke plant workers.

Future activities will depend in part upon leads uncovered in the analyses of the morbidity and occupational mortality data. Future studies can utilize unique local sources of information such as the "population laboratory" in the Arsenal Health District, the one percent city sample and the mass chest x-ray survey of 1953. This line of work in Pittsburgh offers great potential and will lead to even closer collaboration with the Departments of Occupational Health and Biostatistics of the School of Public Health.

Collaboration with the University of Pittsburgh Graduate School of Public Health has identified another area in which the two institutions can profitably join forces. The Biometry Branch, NCI, has many study materials suitable for dissertation topics and other research publications but inability to recruit suitable staff in sufficient numbers precludes exploitation of the data to maximum advantage. The Department of Biostatistics of the School of Public Health is in a position to recruit graduate students but lacks dissertation topics. A difficulty with graduate training in biostatistics, particularly those phases concerned with observational data, is the need for experience with real data which requires guidance by a preceptor. The mutual needs of both institutions have been served by extending faculty appointments to some members of the Biometry Branch. All appointments are at the grade of Associate Professor or above, permitting the staff members to be responsible for doctoral candidates, so that residence at the Biometry Branch is equivalent to residence at the University of Pittsburgh. The shortage of biostatisticians continues to be a critical bottleneck in the development of many medical research programs in the United States and participation in a graduate training program will help meet pressing national needs.

Serial No. NCI-4258
1. Biometry Branch, OASDD, Etiology
2. Demography Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Staff Studies of Cancer in Human Populations

Previous Serial Number: Same

Principal Investigator: John C. Bailar, III

Other Investigators: Haitung King, Zelda Janus, John L. Young, Jr.

Cooperating Units: University of California Medical School
New England Deaconess Hospital
Lahey Clinic
Connecticut Tumor Registry
Laboratory of Pathology, NCI

Man Years:

Total:	7.5
Professional:	4.5
Other:	3.0

Project Description

Objectives:

To describe cancer morbidity and mortality in human populations; to seek out and examine significant differences between persons who develop cancer and those who do not; to search for clues to causative or promoting factors of cancer by comparisons between high-and low-risk populations; to relate epidemiologic features of cancer to survival rates; to identify personal and environmental factors of potential significance in the prevention, control, early diagnosis, or successful treatment of cancer; and to present the results in an intelligible and usable form to medical students and practicing physicians as well as to other research workers.

Methods Employed:

These studies require an extremely wide variety of methods of data collection and analysis, and in many cases new methods must be developed. Where appropriate, these are described in the following sections.

Major Findings:

1. World maps of cancer incidence, mortality, and relative frequency patterns have been completed and an accompanying text written. The maps generally confirm prior impressions. The most interesting unexpected findings are: a) a broad belt of high rates for esophageal cancer, extending from Northern China across Mongolia and the U.S.S.R. to Iran, b) high risks for cervical cancer among North African Jews, in contrast to very low rates among Jews elsewhere, and c) high risks for nasopharyngeal cancer in Morocco, Algeria, Tunisia, and Malta. This work will soon be published.

2. Staff members have been active in advisory and consultative services. These include refereeing and reviewing papers for the Journal of the National Cancer Institute and many other journals, consultation to outside committees, service on dissertation committees (Yale University Department of Public Health and Preventive Medicine) for students working on problems related to cancer, and very frequent advice to other NCI staff members on statistical and epidemiologic problems.

3. A prospective study of approximately 4,000 Lutheran ministers has shown that the risk of death from various forms of cancer and other diseases differs from that in the general population. The differences are being investigated for their etiologic implications and the study has been extended to three other denominations. In general, clergymen have been found to have extremely low total mortality rates.

4. This work is now being extended to 4 other groups of clergymen of other denominations and, presumably different demographic characteristics.

5. Regular surveillance of cancer incidence and mortality rates, both here and abroad, has been continued. A detailed study of cancer mortality in 1959-61, supplementing and extending the report by Gordon, Crittenden, and Haenszel (NCI Monograph No. 6) has been completed but will not be published until we can add the 1964-1966 mortality data.

6. A study of the time lag between cases of leukemia in various townships of Connecticut has been completed. If leukemia is in some way transmitted from one person to another, one might expect an increased number of cases to occur at intervals corresponding to the latent period of the disease; no such increase was found. A medical report on these results is being prepared, and a theoretical paper on new analytic methods required for the analysis is in progress.

7. The Biometry Branch has acquired copies of death certificates for over 2000 persons who died of cancer of the pharynx during 1963-64, and is writing to the physicians who signed these death certificates to ask for additional information on the site of the cancer. Since at present over half of all death certificates for pharyngeal cancer do not even specify whether the tumor was in the nasal, oral, or laryngeal part of the pharynx, this study will permit the first detailed mortality study of the disease.

8. A study of cancer in American Indians, undertaken with Dr. Lucia Dunham (NCI-LP) and Dr. Gert Laqueur (NIAMD) has been postponed because of Dr. Laqueur's illness.

Significance to the Program of the Institute:

This program helps establish baselines for cancer mortality and morbidity in various populations from which changes can be measured, measures trends in cancer rates which may reflect the action of various etiologic agents, identifies high- and low-risk population groups which can be studied and compared, indicates areas for intensive efforts at case finding and follow-up, and provides for the correlation of laboratory studies of cancer with observations on human populations. Ad hoc investigations are undertaken because of unusual or unexpected findings from the routine monitoring of incidence and mortality rates.

Work under this and related projects provides a tie between epidemiologic and survival studies of cancer. The use in epidemiologic investigations of data originally collected for survival studies (prostatic and bladder cancer surveys) and the reverse (Connecticut-Southwest Region cervical cancer study) are both leading to interesting and potentially significant results with much less cost in time and effort than if separate studies had been undertaken.

A new survey of cancer incidence and mortality in the United States is planned for the three years around the 1970 census.

The projects now in progress or recently completed reflects the particular interests of staff members. Substantial progress has been made in several areas but a vast array of other important problems will remain untouched until interested and qualified investigators are available. This is especially true since there has been a very heavy drain of Demography Section personnel to work on the new cancer survey (see NCI-).

Proposed Course of the Project:

It is difficult to predict the course of this project because many ad hoc studies are undertaken on short notice, as their need becomes apparent and as staff time is available. The coming fiscal year should see the completion of a number of specific studies already in progress as well as further development of certain general program areas:

1. The series of reviews of the literature on various aspects of cancer epidemiology will continue. In the coming year these may include those based on geography (a study of cancer incidence and mortality in the Soviet Union), on site of cancer (esophagus, perhaps oral cavity) and on the economic effects of cancer. The reviews on cancer in the Soviet Union and on esophageal cancer are well started, but have been delayed because of a lack of time. In addition we are beginning to plan for a revision of Cancer Rates and Risks, a short book for practicing physicians, medical students, and others, that has been very popular since it was published in 1964.

2. Analysis of hospital records and other documents for approximately 1,800 patients with esophageal cancer is in progress. This is the first review of this disease based on a defined population and should provide useful information of the epidemiology of esophageal cancer and the prognosis for defined groups of patients. The two items receiving earliest attention are the characteristics of the (very few) three-year survivors, and differences in the epidemiology of cancer in various parts of the esophagus.

3. Hospital records of approximately 800 patients with multiple myeloma have been abstracted and the data are being analyzed. This will be the first such study based on defined population, and should answer many questions about the epidemiology and survival patterns of the disease.

4. The analysis of mortality of clergymen will be continued. There is a reduced risk of death in all age groups and for most major causes of death, including cancer, but the reasons for this reduced risk are not yet known.

5. Three earlier follow-up studies are being repeated in order to extend the period of observation: An examination of late mortality among women who have survived at least five years after the diagnosis of cancer of the uterine cervix or corpus; a study of long-term mortality in relation to the dose of radiation given for cancer; and a survey of rectal cancer incidence rates among persons with and without adenomatous polyps of the rectum.

6. Theoretical statistical investigations on the optimum doses of toxic drugs will be continued, with further study of four models already considered and the development of new models.

7. Work will continue on the processing of relative frequency data for approximately 800 cancers of American Indians which have been microscopically confirmed at NIH (Dr. Gert Laqueur, NIAMD).

8. The Demography Section will continue supplying consultation and other assistance to two NIH grantees in the Republic of South Africa:

Dr. Elizabeth Rose, studying esophageal cancer among the Bantu, and Dr. Paul Keen, studying general cancer incidence patterns among the Swazis.

9. An extensive review of the literature on mortality from cancer and other diseases in relation to religion has been accepted for publication.

10. The first paper on cancer epidemiology, by Domenico Rigoni-Stern, has been translated from Italian into English and has been accepted for publication.

11. A study of the survival of old (age 75 and over) women with breast cancer is complete; results indicate a very good prognosis for these patients in relation to their age. This paper is now ready for submission.

12. The geographic distribution of urinary bladder cancer within Connecticut is being investigated, and a manuscript should be finished in a few months.

13. An interview study of patients with cancer of the prostate and urinary bladder will continue for six months more before we attempt any analysis. So far, approximately 930 cases and matched controls have been interviewed and the records coded.

14. Extensive data on cancer epidemiology have been collected and assembled in a uniform way for submission to Dr. Juan del Regato to use in the new revision of Cancer, by Ackerman and del Regato. They can use only a small part of what we have collected, so we plan to publish the data separately.

Honors and Awards

Bailar, John C., III: Invited panelist, Ninth International Cancer Congress. Lecturer (biostatistics), Yale University. Invited paper, Scientific Conference on Cancer in Africa, Nairobi, January, 1967.

King, Haitung: Continued to serve as Clinical Associate Professor of Community Medicine and International Health, Georgetown University Medical School and as Advisor-Consultant, D. C. Interagency Council on Smoking.

King, Haitung and Bailar, John C., III: Invited paper, Annual Meeting of Population Association of America, April, 1968.

Publications

Bailar, John C., III: Nasopharyngeal cancer in white populations--A world-wide survey. UICC Monograph Series, 1: 18-23, 1967.

Bailar, John C., III: Race, environment, and family in the epidemiology of cancer of the nasopharynx. UICC Monograph Series, 1: 101-105, 1967.

Bailar, John C., III: Cancer of the Cervix, the Corpus, and the Uterus. Medical Digest 13: 77-82, March, 1967.

Bailar, John C., III and Gurian, Joan: The Medical Significance of Date of Birth. Eugenics Quarterly 14: 89-102, June, 1967.

Bailar, John C., III: The Epidemiology of Nasopharyngeal Cancer. Proceedings of a panel at the 9th International Cancer Congress, Tokyo, 1966. (In press)

Bailar, John C., III: Epidemiology Studies on Cancer-Reports at the 9th International Cancer Congress, Tokyo, 1966. Cancer Research 27: 819, 1967.

Janus, Zelda L., Bailar, John C., III, and Eisenberg, Henry: Blood Group and Uterine Cancer. American Journal of Epidemiology 86: 569-578, 1967.

Scotto, Joseph, and Bailar, John C., III: Rigoni-Stern and Medical Statistics - A Nineteenth Century Approach to Cancer Research. J. History of Medicine. (In press)

King, H., Bailar, John C., III: Mortality Among Lutheran Clergymen. Milbank Memorial Fund Quarterly. (In press)

Serial No. NCI-4260
1. Biometry Branch, OASDD, Etiology
2. Demography Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Statistical Consultation for the Veterans Administration Urological Research Group

Previous Serial Number: Same

Principal Investigator: John C. Bailar, III

Other Investigators: Robert Huse, Ronald Ferguson, Susan Devesa

Cooperating Units: V.A. Urological Research Group (18 hospitals)

Man Years:

Total:	5.5
Professional:	3.0
Other:	2.5

Project Description

Objectives:

To provide consultative services in statistical and epidemiological methodology necessary for the various programs sponsored by the Veterans Administration Urological Research Group. This includes assistance in the design, interpretation and evaluation of the clinical trials conducted by the Veterans Administration Urological Research Group, and provision for studies operating concurrently with the clinical trials such as epidemiological studies.

Methods Employed:

Standard methods of biometry, statistics, probability, and epidemiology with necessary modification as required by the particular problem. New techniques are developed by the personnel working with the Section to handle specific problems.

Major Findings:

This Section assists the investigators in developing and evaluation methods of treatment for cancers of the male genitourinary tract. Such assistance is generally acknowledged in the publication of findings of the medical investigators. New statistical techniques are published independently.

The Section is more deeply involved in four separate clinical trials:

A. Main Prostate I, which compares the effect of two treatments (prostatectomy alone, and prostatectomy with estrogen) for Stages I-II, and of four treatments (estrogen, orchiectomy, estrogen plus orchiectomy, and placebo control) for Stages III-IV. The intake of new patients into this study was terminated on March 31, 1967. About 2300 patients had been entered by that date.

B. Main Prostate II, which also compares the effect of two treatments (prostatectomy alone and placebo control) for Stages I-II and of four treatments (three levels of estrogen and placebo control) for Stages III-IV. This study is designed as a sequel to Main Prostate I, and was opened to new patients on April 1, 1967. About 250 patients had been entered by April 1, 1968.

C. Focal Carcinoma, which compares several treatments for very early prostatic lesions of the type commonly found in TUR or autopsy specimens from old men. There are now 147 patients in this study.

D. Urinary Bladder, which compares surgery alone, radiation alone, and combined therapy for bladder cancer. There have been 56 patients admitted.

In addition to these four clinical trials, the VA group has several other studies for which NCI provides consultation as requested.

The data from three trials - Main Prostate II, Focal Carcinoma, and Urinary Bladder - are not yet sufficient for detailed study.

The results of Main Prostate I have already caused widespread changes in the general treatment of prostatic cancer, because of the finding that the standard estrogen treatment for prostatic cancer carries a very great risk of death from cardiovascular diseases. In addition, the clinical benefits with respect to the cancer are smaller than was generally believed. Overall mortality rates in Stages I, II, and III, and in "early" Stage IV are substantially higher in estrogen-treated patients than in those not receiving estrogens. This hazard was not recognized before the results of this study were available.

During the past year, efforts in this project have been concentrated on three objectives, dissemination of the results on survival with various treatments, elucidation of the mechanisms by which estrogen exerts its harmful effects, and classification of patients with respect to the probable benefits or losses associated with each treatment tested.

With respect to the first, the results of the VA study have been very widely discussed (not always accurately) among practicing urologists. I understand that one of the examiners for the American Board of Urology recently asked 22 board candidates about this study and found that all of them knew the most important findings regarding the risk of estrogen treatment.

Our efforts at elucidation of mechanisms have so far met complete frustration. At the time that Main Prostate I was organized there was no reason to suspect an adverse effect of estrogen on the cardiovascular system so much important information was not collected and submitted in a uniform way. We are now attempting to gather some items retrospectively (pre-treatment EKG, history of heart disease, admission blood pressure, etc.) but the analysis will be difficult. Main Prostate II includes very careful study of the cardiovascular system before treatment and at each follow-up, and may eventually provide answers to this problem.

Classification really involves two problems - prediction of the (good) effects of treatment on the cancer, and prediction of the (bad) effects on the cardiovascular system. We now have a simple and very accurate way to measure the probability of death from cancer within the first three years after treatment, and this method can be extended to cover later intervals when we have more long-term follow-up data. Prediction of bad effects has been much more difficult, and we do not yet have a good system for identifying a group of patients who are likely to have an adverse reaction to estrogen.

It must be noted that cardiologists have not accepted the estrogen findings as readily as urologists. Results of the VA study have been of concern to the National Heart Institute because of its involvement in the Coronary Drug Project in which several drugs (including estrogen) are actually being used in the hope of reducing mortality among men with a recent myocardial infarct. NHI has assigned a staff member to make a detailed review of our original records and other data on Stages I-II, where the adverse effect of estrogen is most marked. His review has just been completed, and completely supports all of our published conclusions. His report should be published soon.

Significance to the Program of the Institute:

This Section serves the members of the Veterans Administration Urological Research Group by assisting in preparing and revising protocols, forms, and records; giving statistical guidance in the design and execution of clinical trials; processing and analyzing data that is received daily on patients in the various studies; and assisting the members of the Group with reports on the progress of the various studies.

In return for these services, the Demography Section has access to the largest complete set of data available anywhere on the course of prostatic cancer. Over 2300 patients have been entered in Main Prostate I with follow-up as long as eight years. These data are proving extremely useful and interesting not only in the comparison of treatment effects, but also in defining the general behavior of prostatic cancer in relation to a host of personal, clinical, and laboratory characteristics.

Proposed Course of the Project:

The cooperative studies undertaken jointly by the Veterans Administration and this Section are planned to be a self-generating sequential series to continue as long as fifteen years. Consultative services of this Section have expanded considerably over the past year as the data from Main Prostate I have ripened, and there will undoubtedly be further expansion as these patients move into the later phases of this very slow disease, and as patients are accumulated in Main Prostate II.

In the coming years, we will continue to give most of our attention to the analysis of Main Prostate I, and especially of the patients in Stages III-IV, since the other studies do not yet include large numbers of patients with long-term follow-up. In particular, we plan to continue a detailed microscopic classification for as many patients as possible with frequent study of all data at hand.

Studies of specific cardiovascular effects of estrogen on patients in Main Prostate I have been undertaken, but the data will be scanty and difficult to interpret. Main Prostate II has been designed to include detailed heart studies, but it may take several years to collect sufficient data for a full analysis.

The great body of detailed clinical information collected in the project is already proving to be useful in ways not related to treatment evaluation. A statistical "profile" of prostatic cancer patients is being compiled; surgical mortality is being investigated; studies are in progress to determine the prognostic significance of a change in acid phosphatase level, appearance of osteolytic or osteoblastic bone metastases; the order in which symptoms develop and the time intervals between symptoms are being studied; etc.

Epidemiologic and demographic studies derived from this group are reported under Serial No. NCI-4258.

Honors and Awards

Bailar, John C., III: Invited papers, American Radium Society and American Urological Association (Mid-Atlantic Section).

Publications

Mellinger, George T., Gleason, Donald, and Bailar, John C., III: The Histology and Prognosis of Prostatic Cancer. J. Urol. 97: 331-337, 1967.

The VA Cooperative Urological Research Group: Carcinoma of the Prostate: Treatment Comparisons. J. Urol. 98: 516-522, Oct., 1967.

The VA Cooperative Urological Research Group: Treatment and Survival of Patients with Cancer of the Prostate. Surgery, Gynec. and Obstet. 124: 1011-1017, May, 1967.

Bailar, John C., III, Becker, Leslie H., and Parry, William L.: Factors in the Prognosis of Carcinoma of the Prostate - A Cooperative Study. J. Urol. (In press).

Serial No. NCI-4254
1. Biometry Branch, OASDD, Etiology
2. End Results Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on Cancer in a Defined Population (Connecticut)

Previous Serial Number: Same

Principal Investigators: Sidney J. Cutler

Other Investigators: William I. Lourie, Jr., Herman Heise, Lillian Axtell, Roger Connelly, Max Myers

Cooperating Units: Connecticut State Department of Health

Man Years:

Total:	7.5
Professional:	4.0
Other:	3.5

Project Description

Objectives:

To provide data on cancer incidence, methods of treatment, and end results; to investigate the effect on incidence of factors such as urban environment, nativity, economic status, occupation, diet, and personal habits; and to study in detail the inter-relationship between the condition of the patient at diagnosis, the therapeutic course, and the end results, in the general practice of medicine.

Methods Employed:

1. Hospitals accounting for all but 5 percent of the approved general hospital beds in the State participate in a case reporting and follow-up program administered by the Connecticut Health Department. Samples of cases reported to the register are drawn for special study involving the examination of hospital records for additional clinical details, reexamination of histologic specimens, and the collection of histories from patients and relatives.

2. In collaboration with staff of the Connecticut Tumor Registry, work is nearing completion on a monograph on trends in treatment policy and in patient survival.

3. Upon completion of the above-mentioned monograph, a number of site-specific analyses will be carried out relating trends in incidence rates to trends in stage of disease at diagnosis and patient survival.

Major Findings:

The most important trends in the incidence of cancer through calendar year 1962 were: (1) A sizable increase among males in the incidence of all forms of cancer combined during the past 25 years; in females the increase has been moderate since 1950. (2) A sharp and continuing decrease in the incidence of cancer of the stomach in both sexes. (3) The decrease in stomach cancer incidence has been accompanied by a sizable increase in the incidence of colon cancer, but little change in the incidence of rectal cancer. (4) The marked increase in the incidence of cancer of the lung in men is continuing. (5) The introduction of the vaginal smear has resulted in a decrease in the incidence of invasive carcinoma of the cervix. (6) A number of other forms of cancer have increased in frequency of occurrence: breast in women, uterine corpus, prostate, kidney, bladder, lymphomas, and leukemia.

Significance to the Program of the Institute:

The Connecticut Cancer Registry is currently the only population-based program in the United States which provides extensive, reliable information on cancer incidence and mortality and information on the end-results of treatment. The completeness of case reporting and the efficiency of patient follow-up make possible the evaluation of the impact of case findings and treatment programs on the cancer problem as it affects a defined population. It provides a continuous resource for the study of cancer epidemiology and of the influence of social and biological factors on the end-results of treatment. It also provides a means for estimating costs and evaluating analyses of the larger series of data obtained via the End Results Program.

Proposed Course of Project:

In order to more fully exploit the data and the resources of the Connecticut Tumor Registry, Roger Connelly of the Biometry Branch has been detailed to Connecticut. Upon completion of the two monographs that he is currently working on, plans will be developed for a variety of special studies requiring the collection of more comprehensive data through patient and family interviews and through mailed questionnaires, as well as through review of hospital records.

Publications:

Connelly, R.: Cancer mortality among the foreign born in Connecticut. Connecticut Health Bulletin 81: 10, October, 1967.

Eisenberg, H. and Connelly, R.: Some preliminary data on the postmastectomy treatment of female breast cancer in Connecticut. Connecticut Medicine 31: 193-198, 1967.

Cancer in Connecticut - Incidence characteristics, 1935-1962. Connecticut State Department of Health, 1967.

Serial No. NCI-4257
1. Biometry Branch, OASDD, Etiology
2. End Results Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Collaborative Studies of Cancer in Human Populations

Previous Serial Number: Same

Principal Investigators: Sidney J. Cutler, William Haenszel

Other Investigators: William I. Lourie, Jr., Lillian M. Axtell, Herman W. Heise, Roger R. Connelly, Max H. Myers, Joseph Scotto, John W. Berg

Cooperating Units: California Tumor Registry; Connecticut Tumor Registry; Massachusetts Cancer Register; Albert Einstein Medical Center, Philadelphia; Charity Hospital of Louisiana; Indiana University Medical Center; New York Medical College; State University of Iowa Hospital; University of California Medical Center; University of Chicago Clinics; University of Michigan Hospital; University of Virginia Hospital; Norwegian Cancer Register; Unit on Epidemiologic Pathology, Tohoku University; University of Hawaii; California Department of Public Health; University of Arizona; University of Colorado

Man Years:	
Total:	9.5
Professional:	4.5
Other:	5.0

Project Description

Objectives:

To assist other Branches of the National Cancer Institute, other health agencies, and treatment centers in the development and maintenance of programs for the collection and analysis of data on cancer in human populations and on the end results of treatment. This includes consultation and advice on methodological problems, development of improved statistical tools for the analysis of observations on human populations, and active collaboration in the collection and analysis of data in specific projects.

Methods Employed:

1. The consultation draws on standard statistical and actuarial techniques and accepted principles for records management, with necessary modifications required for any individual study situation. Many of the problems can be handled by correspondence, but field trips are frequently required. Consideration must be given to the study objectives of the individual investigator or agency, the existing administrative arrangements, and the personnel and financial means at hand to implement the program of operations and studies.

Most of the consultation work in this area concerns problems in the integrated use of data from various study techniques: analyses of mortality data, controlled retrospective and prospective studies of selected cohorts, morbidity surveys, screening of populations for precursive signs, and cancer registers. The Branch staff is particularly interested in the methodological problems and issues involved and will continue to do research in this area.

2. Field work in Japan, Hawaii, and California on the study of shifts in risk for cancer of the stomach and lung among migrants from Japan to Hawaii and California proceeded according to plan. In Japan, the principal collaborator is Professor M. Segi, Tohoku University, and work in Hawaii and California is carried out under the direction of Dr. Richard K. C. Lee, School of Public Health, University of Hawaii and Dr. John E. Dunn, Jr., California Department of Public Health. Editing and coding of records from all study areas is done in Japan in Professor Segi's department. The resulting punch cards and related documents are transmitted to the Biometry Branch, National Cancer Institute, which has primary responsibility for the tabulation and analysis of data.

The punch cards for the household surveys in Miyagi and Hiroshima prefectures, Hawaii and California have been received by the Biometry Branch. The initial paper summarizing the data with emphasis on measures of variation within Japan will be published in the Tohoku University Journal of Experimental Medicine. This will be followed by a paper reporting on the demographic and cultural characteristics, including diet patterns, for Japanese in Hawaii and California in relation to the background data for Japan.

The interviews of stomach cancer patients and matched hospitals controls has been completed in Miyagi and Hiroshima prefectures. Case-control interviews continue in Hawaii and California and will be accumulated until a number sufficient for preliminary analysis have been assembled. Besides yielding descriptive population data the household interviews provide a set of general population controls for the stomach-cancer series. Some preliminary results from the case-control interviews for stomach cancer in Miyagi prefecture were reported by Mr. Haenszel at the panel discussion on stomach cancer at the Ninth International Cancer Congress. The stomach cancer case-control series in hand from Miyagi and Hiroshima prefectures is being reviewed, but active analysis of the results awaits the findings from the comparison of the household survey data from Hawaii, California and Japan. These data will provide the base for a paper examining the pattern of relative risks of stomach cancer within Japan with respect to a number of demographic and cultural variables, and attention will be directed to variations in risk by degree of adherence to or deviation from the traditional Japanese diet. These results will be augmented by a first analysis of the corresponding case-control series collected in Hawaii.

In Miyagi prefecture and in Hawaii detailed pathology protocols have been completed for stomach cancer patients interviewed when operative or necropsy specimens are available. This work is under the direction of Dr. K. Herrold, Dr. G. Stemmermann, and Professor K. Akazaki.

A series of summary tabulations was distributed to the participating pathologists in preparation for their April 1967 meeting and the information taken into account in arrangements for special comparative reviews being undertaken by Drs. Sasano and Stemmermann. A noteworthy feature of the present study is the close integration of demographic, epidemiologic, and pathology data for the same series of patients.

The program of studies for Japanese migrants began with two sites -- stomach and lung. The intent was to continue work over a period of years and to phase in protocols for new sites as work for sites under investigation was terminated. The series of stomach cancer cases in Japan has attained the size desired and work in Japan on this site was discontinued in 1965. Interviews for background data on stomach cancer, however, must continue for several more years in Hawaii and California. Interviewing in Japan for additional sites (esophagus, colon and rectum, leukemia) was started in 1966, accompanied by parallel collection of data in Hawaii and California. With the collaboration of Dr. John Berg, pathology protocols have been developed for colon, rectum, and esophagus for use in all three study areas.

A combined epidemiologic-pathology approach will be pursued. For example, prostatic cancer is a site displaying interesting features among Japanese migrants. However, comparative studies of clinically manifest prostatic carcinoma are difficult to pursue until the status of occult prostatic carcinoma among males in Japan can be resolved. Dr. Berg has arranged with Dr. Akazaki for a comparative review of a series of prostate specimens for necropsies of white males (Veterans Administration Hospital) and of males in Aichi prefecture using the same criteria and methodology for both.

3. The Biometry Branch is collaborating with the National Heart Institute, the London School of Hygiene, and the Cancer Registry of Norway in a study of cardiorespiratory diseases and cancer among English and Norwegian migrants to the United States. The background and design of these studies has been described by Reid (National Cancer Institute Monograph No. 19, pps. 287-299). The Bureau of the Census designed the sample and interviewed a roster of about 50,000 migrants from England and Norway and obtained a list of all living sibs still residing in those countries. The conduct of field work in the United States has been described by Pearl, Levine, and Gerson (National Cancer Institute Monograph No. 19, pps. 301-320). Mortality follow-up of the study

groups in the United States has been handicapped by insufficient staff. However, three years experience has been accumulated and a report analyzing mortality differentials for specific cancer sites and cardiovascular diseases by age at time of arrival in the United States and duration of residence in this country will be completed in the ensuing years.

The information on prevalence of cardiorespiratory symptoms among the U. S. study populations has been reported in a preliminary communications by Reid, et al (National Cancer Institute Monograph No. 19, pps. 321-346). A striking feature of the symptom data was the low prevalence of respiratory symptoms among British migrants in the United States whose experience was comparable to that for the U. S. native-whites and far below the prevalence rates reported in the United Kingdom. The symptom prevalence for British migrants varied only slightly with respect to age at time of migration and place of birth and residence in the United Kingdom. The symptom data were consistent with the evidence provided by mortality data. It is worth noting that the behavior of chronic non-specific lung disease among migrants is much different than the picture presented for lung cancer.

Collection of comparable demographic and symptom data in England and Norway from samples of the general population and from the sibs of migrants began in March 1963. The field work has been completed in both countries and the editing and coding of schedules and the preparation of punch cards and computer tape was completed late in 1966. Arrangements for the collection of death certificates for persons in the study cohorts have been made and the intention is to trace all deaths forward in time from January 1965 in England and Norway. Collation on computer tapes of the data for the index migrants in the U. S. samples and for their living sibs in Norway has been completed. Work is being financed by a NCI research contract and in England by a NHI grant.

4. Provide staff support to the End Results Group, which represents more than 100 hospitals participating in a cooperative program for the evaluation of the end results of cancer treatment. This included advice on the development and applications of uniform definitions and procedures for participating cancer registries, consultation on planning and implementing special studies, editing and analysis of data submitted to the National Cancer Institute in accordance with a Uniform Punch Card Code, and exploration of more efficient data processing and statistical techniques to abstract the maximum amount of useful information from the collected data. In addition, leadership is provided in establishing working relationship with cancer registry program in other countries.

The End Results Evaluation Program provides a baseline for planning and for interpreting controlled therapeutic trials, and for evaluating results reported by specialty centers.

The resources of the End Results Group provide a continuing opportunity for evaluating and testing the recommendations of the American Joint Committee on Staging and End Results Reporting. Liaison with this committee is facilitated by the fact that Dr. Cutler is a consultant to this committee and regularly attends committee meetings.

The data collected through the End Results Group (currently 40,000 new cases per year) provide a basis for evaluating trends in patient characteristics, stage of disease, treatment practices, and associated end results in the general run of patients. A major contribution is made by Connecticut, which is a population-based registry in Alameda County; and Massachusetts, which is developing a population-based registry in the Boston area. The population-based registries make possible evaluation of trends in patient survival in the light of trends in cancer incidence. The wide variety of institutions participating in the program make possible comparison of experience in university and non-university hospitals, government supported (county and city) and privately supported hospitals, large and small hospitals. In addition, this broad data resource makes possible the selection of specific series of cases for detailed study through review of hospital records. Of particular advantage is the availability of sufficiently large numbers of cases for study of relatively rare forms of cancer.

Collaboration with cancer registries in other countries is facilitated in part through the Ad Hoc Group on International Cooperation in Evaluation of End Results. Dr. Cutler is the Executive Secretary of the Group. Broadened opportunities for collaboration with registries in other countries should develop through the organization of an International Association of Cancer Registers, which is now in progress. Dr. Cutler proposed the establishment of such an association at a meeting of cancer registry representatives during the course of the Ninth International Cancer Congress in Tokyo. Mr. Haenszel is Chairman of the organizing committee.

5. Statistical analysis is under way on the study of the prognostic significance of morphologic tumor characteristics in cancer of the breast. The study is based on a review of slides in Norway by Dr. Maurice Black of New York Medical College.

6. A retrospective detailed review of hospital records on approximately 2,000 leukemia patients has been initiated under the direction of Dr. Calvin Zippin of the University of California Tumor Registry. The study is designed to identify factors which may be associated with the variation in patient survival rates with respect to age. For example, among patients with acute lymphocytic leukemia, there is a correlation between the initial white cell count and the marked inverse gradient of survival with age. Information on treatment and response to treatment, as well as on initial signs, symptoms, and laboratory findings is being collected.

Major Findings:

1. The up-dated punch card decks from the members of the End Results Group, which include newly diagnosed cases through 1964 and follow-up through 1966

have been used to prepare a comprehensive report (End Results in Cancer -- Report No. 3) for distribution at the Sixth National Cancer Conference in September 1968.

2. Institutions participating in the End Results Evaluation Program treated more than 10,000 newly diagnosed cases of leukemia during the period 1940-62. Analysis of this very large series revealed trends and relationships that may be missed or may be unclear in series of a few hundred cases seen in one treatment center. Some of the findings were:

a) There has been a marked increase in the percentage of leukemias classified as acute. This increase occurred in all age groups and for both lymphocytic and myelogenous leukemias. Review of a sample of slides is required to determine whether this simply reflects changes in diagnostic criteria. A true increase in the relative frequency (and incidence) of the acute leukemias would be important in the epidemiologic study of the disease.

b) Although generally there was a continuing increase in the use of chemotherapy, the increase varied with the morphologic type of disease and the patient's age. Detailed review of patient records is needed to determine whether the less frequent use of chemotherapy in adult and elderly patients is related to ability to tolerate toxicity.

c) The variation in the age distribution for the different types of leukemia is brought out very clearly in this large series. For example, in patients under 20 years of age, acute lymphocytic leukemia occurs most frequently at ages 3 and 4. In contrast, the age distributions for acute myelogenous and monocytic leukemia are essentially flat.

d) The median survival time for the totality of patients with leukemia increased from 3.3 months in 1940-49 to 6.9 months in 1955-62; an encouraging increase, but prognosis remains poor. Even in children under 10 years of age with acute lymphocytic leukemia, the group exhibiting the most dramatic improvement, median survival time in 1960-62 was 11 months. This latter figure is in line with reports from a number of specialty centers that reported results for unselected series . . . . Although increase in survival time occurred in all age groups, from 1955-59 to 1960-62 there was a reversal in the upward trend among middleaged and elderly patients. The reversal occurred primarily among patients with chronic lymphocytic leukemia. A detailed record review to identify factors associated with this reversal is under way.

3. Detailed review of a sample series of 2,039 breast cancer cases may lead to modification of concepts regarding the "staging" of cancer. This study points to a need for distinguishing between the criteria for a summary classification of "extent of disease" to facilitate reporting of therapeutic results and a descriptive system to help the clinician select a treatment plan . . . . . The study also uncovered some interesting implications concerning the relationship of clinical and pathologic evaluation of extent of disease. Whereas the pathologists' evaluation of axillary node involvement is of dominant

prognostic significance, the clinician's assessment contributes useful information. Among patients with pathologically positive nodes, mortality was generally higher in patients with clinically palpable nodes than in patients with clinically negative nodes. In contrast, among patients with pathologically negative nodes the prognostic significance of clinically palpable nodes seem to depend on the size of tumor. In the presence of a small tumor, clinically palpable nodes were associated with higher mortality. In the presence of an intermediate sized tumor, there was no difference in mortality between patients with and without clinically palpable nodes. In the presence of a large tumor, a reversed relationship was observed, i.e., clinically palpable nodes were associated with lower mortality. Thus it appears that in some patients, particularly evident in the presence of a large tumor, clinically palpable nodes appear to be a manifestation of a defensive host response. Exploration of the relationship of the foregoing observations to pathologic and cytologic characteristics such as nuclear grade and sinus histiocytosis may lead to a better understanding of the biology of breast cancer and may point to the desirability for increased attention to enhancement of host response . . . This study also has implications in regard to suggestions for a clinical trial comparing simple with radical mastectomy. The clinician's evaluation of extent of disease, a basic consideration in planning treatment is frequently unreliable. Even in the most favorable group on clinical criteria (clinically negative nodes, small tumor, no fixation to skin or underlying tissues), 21 percent were found to have axillary node metastases by the pathologists.

Significance to the Program of the Institute:

Many of the benefits of the consultation program are long range in nature and consist of setting up record systems and study situations which enlarge the potential study resources of NCI and related organizations. The research objectives of the Institute are promoted by continued work on the integration of various study techniques, e.g., retrospective and prospective studies, analyses of mortality data and morbidity surveys, epidemiologic investigations and cancer registers. An example of the interaction between epidemiologic and end-results investigations are the developing studies of prognostic factors and the role of these factors in cancer etiology.

Proposed Course of the Project:

1. With the publication of the revised manual of procedures, definitions, and codes, emphasis will be placed during the coming year on helping the participating registries adjust their local operations to comply with the revised requirements.

2. Work will proceed on several special studies, but major emphasis will be placed on the preparations of a series of analytical and interpretive papers based on data pertaining to 425,000 patients diagnosed during the 25 year period 1940-1964. These data have been reported on in End Results Report No. 3 to be distributed at the Sixth National Cancer Conference in September, 1968. The projected series of papers will contain more interpretive material and will

be submitted to a variety of scientific journals.

3. A close working relationship is being worked out with the Epidemiologic Pathology Unit under Dr. John Berg. A preliminary review of End Results Group data reveals that the information regarding histologic type warrants improvement both with respect to comparability among registries and the accuracy of the reported information. Dr. Berg and his associates can be of great help in clarifying the nomenclature and in improving the quality of the reported data. They will also play an active part in planning and implementing special studies. For example:

1) in determining the extent to which the reported increase in the relative frequency of acute leukemia is due to changes in diagnostic criteria; and

2) in studying the biologic implications of the relationship of clinical and pathologic assessment of nodal involvement in breast cancer.

4. During the coming year the possibility of expanding the End Results Group to include cancer specialty centers such as Roswell Park Memorial and M. D. Anderson will be actively pursued. In addition, the possibility of bringing a selected number of V. A. hospitals into the program will be explored. We will continue to explore the possibilities of bringing into the program several additional population-based registries, particularly in the Mid-west and the South.

5. A collaborative arrangement is being developed with the cancer program in Saskatchewan, Canada. This will provide access to a population-based tumor registry which is an outgrowth of a government supported cancer diagnostic and treatment program.

6. The National Cancer Institute remains interested in collaborating with the newly organized Division of Regional Medical Programs and in evaluating the impact of regional programs on cancer control and management. Active collaboration with DRMP in providing guidance in matters pertaining to surveys, record, and statistical activities is being implemented. The potentialities of the Regional Medical Program as a data source will be thoroughly explored, particularly with respect to quality of medical care and evaluation of the results of various patient management programs.

7. The addition to the staff of the Etiology Area, NCI, of Dr. John Berg, Head, Epidemiologic Pathology Unit, Office of the Scientific Director for Etiology, has materially facilitated the planning and execution of studies of prognostic factors and their relationship to the epidemiologic characteristics of cancer. Increased attention will be given to the correlation of histologic and cytologic information with clinical and epidemiologic data.

8. Exploitation of the Norwegian migrant study materials requires access to a population of U. S. born offspring of Norwegian parents which can be followed over time for mortality and morbidity data. For technical reasons this work can be best carried out in the United States in an insured population. With the cooperation of the University of Minnesota School of Public Health, we arranged with the Lutheran Brotherhood, an insurance company with a heavy representation of persons of Norwegian descent among its policyholders, for collection of histories from policyholders (using the same set of questionnaires employed in other phases of the study) and for continued surveillance of death claims. Collection of questionnaires from the policyholders was completed in April 1967 and a response rate of 70 per cent was obtained. Editing of the schedules and coding the returns is underway.

Honors and Awards

Cutler, S. J.: Invited participant in Symposium on Breast Cancer sponsored by the Canadian Cancer Society, March 8 & 9, 1968, Toronto. Invited participant in Symposium on Cancer Registries, sponsored by International Union Against Cancer, May 2-4, 1968, Madrid, Spain. Member, NCI Breast Cancer Task Force; Consultant to American Joint Committee on Cancer Staging and End Results Reporting; Scientific reviewer for Journal National Cancer Institute, Cancer, Cancer Research, and Science.

Publications:

Cutler, S. J. and Myers, M.: Clinical classification of extent of disease in cancer of the breast. J. Nat. Cancer Inst. 39: 193-207, 1967.

Cutler, S. J.: The natural history of treated cancer of the breast. Symposium on Factors Influencing Prognosis of Breast Cancer, Cardiff, Wales, April 12-14, 1967. Proceedings in press.

Cutler, S. J.: Clinical classification of extent of disease, Bulletin, International Union Against Cancer, June, 1967,

Cutler, S. J., Axtell, L. M. and Heise, H.: Ten thousand cases of leukemia; 1940-1962, J. Nat. Cancer Inst. 39: 993-1026, 1967.

Cutler, S. J.: Tumor registries and hospital records. Presented at The American Association of Cancer Education, October 16, 1967. Publication pending in proceedings.

Cutler, S. J.: The natural history of cancer and the assessment of treatment. UICC Symposium on Cancer Registries, May 2-4, 1968. Madrid, Spain.

Cutler, S. J. and Staff: End Results in Cancer, Report No. 3. Publication in press as a PHS monograph.

Cutler, S. J.: The use of tumor registry data. California Medicine 106: 98-107, 1967.

Cutler, S. J., Connelly, R. R. and Eisenberg, H.: The role of a population-based registry in the epidemiology of cancer of the breast. Current Concepts in Breast Cancer, Proceedings of the Symposium on Breast Cancer held July, 1966, at Ochsner Medical Center, New Orleans, Louisiana. pp. 10-18, 1967.

Cutler, S. J., Meyer, M.: Classification of extent of disease in cancer of the breast. Current Concepts in Breast Cancer, Proceedings of the Symposium on Breast Cancer held July, 1966, at Ochsner Medical Center, New Orleans, Louisiana, pp. 41-53.

Cutler, S. J., Heise, H., and Eisenberg, H.: Childhood leukemia in Connecticut, 1940-1962, Blood 30: 1-25, 1967.

Serial No. NCI-4265
1. Biometry Branch, OASDD, Etiology
2. Mathematical Statistics and Applied Mathematics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Consulting in Statistics and Applied Mathematics

Previous Serial Number: Same

Principal Investigators: John J. Gart, Hugh M. Pettigrew

Other Investigators: Howard M. Shapiro, John J. Slivka, Donald G. Thomas

Cooperating Units: None

Man Years:

Total:	4.0
Professional:	2.5
Other:	1.5

Project Description

To provide consulting assistance and collaboration to NCI scientists on the applications of mathematical statistics and applied mathematics to the interpretation, and analysis of scientific experiments.

Objectives:

The principal objectives are (1) to collaborate with NCI scientists on mathematical problems related to cancer research; (2) to provide consulting assistance in statistics and applied mathematics to NCI investigators; and (3) to accelerate the use of quantitative methodology in various aspects of the NCI intramural program.

Methods Employed:

The methodology of applied mathematics, mathematical statistics and probability are applied to biomedical problems. Often variations of existing techniques are developed to suit the special requirements of a particular problem. The members of this section consult with a great many investigators in all areas of intramural research in the NCI. Although two new junior members have been added to the staff, the recent loss of two senior consulting statisticians has greatly increased the per capita load of consulting activities. Thus many of the consulting activities are perforce more limited in the depth and time than is preferable for the more important projects.

Major Findings:

It is difficult to assay the mammary tumor virus using the appearance of tumor as a criterion because of the long latent period. Nandi and DeOme have attempted to shorten the time element by inserting hormone pellets in mice after the injection of the virus and using the number of hyperplastic-alveolar nodules as a measure of potency. In analyzing a series of preliminary experiments, it became apparent that the average number of such nodules was not a good measure of potency. As an alternative it was suggested that the simple presence or absence of at least one nodule in an individual mouse be used. Dr. John Gart, in conjunction with Dr. W. Ray Bryan, Associate Scientific Director for Viral Oncology and Professor S. Nandi of University of California at Berkeley, designed a large experiment involving 1900 mice in six replicates which hopefully will yield a quantitatively precise standard against which future unknown virus preparation may be compared.

Working in conjunction with the members of the Medicine Branch (Dr. S. Perry, D. J. Spivak and Dr. Brubaker), John Gart has devised objective methods of analyzing the disappearance curves of labelled lymphocyte and granulocytes using DFP^{32} and Cr^{51}. It appears the kinetics patterns observed can be used to characterize, to some extent, acute lymphocytic leukemia, chronic lymphyocytic leukemia, and hematological normals. The role of the spleen was also investigated using body surface scanning.

Working with Dr. Preisler and Dr. Hazen of the Medicine Branch, Hugh Pettigrew and John Gart devised a randomization scheme for patient assignment in a new life-island trial.

Hugh Pettigrew analyzed the data from a POMP trial in ALL, which studied (1) the relationship between initial patient status and length of time in remission and (2) the effects of maintenance therapy on length of remission. He also analyzed data relatingthe percent of bone marrow lymphocytosis with length of remission and survival in ALL.

Working with Dr. Preisler of the Medicine Branch, Mr. Hugh Pettigrew has been devising statistical techniques for combining results from several experiments using colony forming units in the spleen.

Hugh M. Pettigrew designed an experimental program to investigate the interaction of long-range, low-level adjuvant chemotherapy with surgery in a mouse mammary tumor system. These experiments, performed by H. Wexler of the Surgery Branch, may have important implications in the treatment of human disease.

In conjunction with Dr. Williams of the Medicine Branch, Hugh Pettigrew, John J. Slivka and Donald Thomas have been setting up the analysis of data on aortic lesions of atherosclerosis in 300 autopsies performed in Nigeria.

Donald Thomas has consulted from time to time with members of the Epidemiology Branch and other members of NCI on the application of statistical methods and computer programming.

Dr. Howard Shapiro working in conjunction with Dr. B. Kronman of the Biology Branch in a study of the purported relationship of delayed hypersensitivity responses to the incidence of neoplasms, particularly carcinoma of the colon. A total of 102 patients and controls from the services of Dr. S. A. Localio of N.Y.U. Hospital were skin tested with four antigens. Preliminary analysis of the data fails to reveal a diminished response in tumor patients, but further analysis is in progress.

A project on a automatic scanning of white blood cell autoradiographs is under development. Howard Shapiro is cooperating with Dr. Lewis Lipkin of NINDB, and R. Kirsch and P. Stein of the NBS on this research.

John J. Slivka has performed valuable work as a consultant with several research groups in the NCI. In particular he developed a simple, but incisive, mathematic model which enabled a simple interpretation of the data of Dr. John Fahey of Immunology Branch on the question of the occurrence of certain pairs of tumors in the same individual. He consulted with Dr. John Marshall of the Endocrinology Branch on a problem in ovulation induction and ovarian enlargement. Other consultations were with several members of the Surgery Branch, the Laboratory of Chemical Pharmacology, and the Medicine Branch on various statistical analyses.

Members of the section have spent a significant amount of effort on refereeing papers on cancer research for publication both within and without NCI. In particular, John Gart has served as referee for *Science* and *The Journal of the National Cancer Institute*, Howard M. Shapiro has served as a referee for *Cancer*, and Hugh M. Pettigrew, for *The Journal of the National Cancer Institute*.

Significance of the Program to the Institute:

Members of the section are assuming an essential role in many programs within the NCI. Their activities include not only statistical analysis but also planning valid experiments sometimes making use of mathematical modesl.

In addition to the interaction with individual scientists and scientific teams, members of the section serve on various committees within NCI. Dr. Gart has served on the Clinical Trials Contract Review Committees and continues to serve on the Chemical Carcinogenesis Contract Review Committee and the Experimental Design Committee of the NCI.

Dr. H. M. Shapiro has served as a member of site visit teams for the Clinical Trials Contract Review Committee.

Proposed Course of the Project:

Several of the projects mentioned in the Major Findings section will continue. In particular, the analysis of the mammary tumor virus experiment will be a major effort. Another project is the construction of a two-stage mathematical model of breast cancer, being investigated in collaboration with Dr. John Berg of Etiology. It is hoped that the section may be able to devote more effort to the investigation of epidemiologic models with Dr. Berg in the coming year. The addition of at least one more statistical consultant would probably be required to implement this project effectively.

Publications

Carbone, P., Kellerhouse, J. E., and Gehan, E. A.: Plasmacytic myeloma: A study of the relationship of survival to various clinical manifestations and anomalous protein type in 112 patients. Amer. J. Med. 42: 937-948, 1967.

Gart, J. J.: Practical scheme for evaluation of total gonadotropins in the urine of prepubertal children. J. Clin. Endocr. 27: 1127-1128, 1967.

Hoye, R. C., Gart, J. J., Weiss, G. H., Riggle, G. C. and Ketcham, A. S.: Potentiation of laser oncalysis with pretreatment X-irradiation. Radiat. Res. 32: 112-116, 1967.

Zelen, M.: A hypothesis for the natural time history of breast cancer. Cancer Res. 28: 207, 1968.

Serial No. NCI-4267
1. Biometry Branch, OASDD, Etiology
2. Mathematical Statistics and Applied Mathematics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Research in Mathematical Statistics and Applied Mathematics

Previous Serial Number: Same

Principal Investigators: John J. Gart, Hugh M. Pettigrew

Other Investigators: Howard M. Shapiro, John J. Slivka, Donald G. Thomas

Cooperating Units: None

Man Years:
Total: 3.5
Professional: 3.0
Other: 0.5

Project Description

Objectives:

To conduct basic research in mathematical statistics, probability and applied mathematics; to develop new statistical methodology which is especially appropriate to bio-medical sciences.

Methods Employed:

The methods employed are the modern theories of mathematical statistics, probability and applied mathematics. High speed electronic computers are often used to compute appropriate mathematical tables and test approximations by simulation techniques.

Major Findings:

The research of the members of this section covers a wide spectrum of topics in mathematical statistics, probability, and applied mathematics. These are summarized below.

1. Tests of the Folded Binomial Distribution. John J. Gart has devised a powerful goodness of fit test for the symmetric folded binomial distribution. This test supersedes an inefficient statistical test suggested by soviet scientists in the radioautographical analyses of

DNA-reproduction in sister-chromatids.

2. Investigation of the Power of the Wilcoxon Test and Modified Wilcoxon Test Against Parametric Alternatives. (D.G. Thomas, E. A. Gehan). This investigation is concerned with the power of the generalized Wilcoxon test and its modification in the presence of censored observations. The underlying survival curves were assumed to be either Weibull or exponential and the nonparametric tests were compared to the F test and a test based on order statistics. The results gave one a good notion as to the relative efficiency of the various tests in comparing survival curves in clinical trials or screening experiments on animals.

3. Linear Models of Cell Biochemistry. H. M. Shapiro has developed an input-output model of a growing cell which gives lower bound estimates on the kinetics of enzyme systems in the cell, using as input a quantitative analysis of cell constituents and the stoichiometry of biosynthetic reactions. Such a model may prove useful in finding differences between various normal and neoplastic cell types which could be exploited in the design of chemotherapeutic agents and schedules.

4. A Treatment vs. Control Median Test. (J. J. Slivka, J. J. Gart.) A quick nonparametric test for comparing the survival curves of several doses of a drug with a control has been devised. This test will prove useful in scanning data for the best dose level and judging its significance.

5. Mathematical Theory of Epidemics. Hugh Pettigrew is continuing research on the stochastic theory of small epidemics in large populations including the case where carriers are present. J. J. Gart has completed work on a deterministic model where two kinds of susceptibles may be found.

6. Small Sample Tests for the Poisson Distribution. (Hugh Pettigrew, John Gart and Donald Thomas). The ratios of the range, sample variance, and higher k-statistics to the sample mean are considered as test statistics of the Poisson distribution. The distribution properties of these test statistics under the null and alternative hypothesis will be investigated.

7. Small Sample Performance of the Complete Ascertainment Estimators. (D. G. Thomas and J. J. Gart). The small sample properties of various estimators of the segregation ratio in the complete ascertainment in family studies is investigated. A computationally simple estimator is found by exact computation of its bias and mean square error to be almost as efficient as the maximum likelihood estimator.

8. Models of Shock (H. M. Shapiro) A hypothesis regarding a molecular mechanism for irreversibility in shock has been formulated. The roles of the cellular sulfhydryl/disulfide ratio and reduction and denaturation of cell protein after prolonged hypotension are important in this model.

9. Results on the Strong Law of Large Numbers (John J. Slivka) The strong law of large numbers guarantees that the sample percentage will tend to the true percentage in an unending series of observations or experiments. If the investigator looks at the data from time to time as it comes in, he may find a large deviation from the expected percentage. This research looks at the probabilities that the series may yield percentages outside of a small neighborhood of the true percentage when the data is "peeked at" repeatedly.

10. Simulation of Mathematical Models. Donald G. Thomas has developed computer programs which simulate the results of clinical trials. These are used to assess the performance of a "Play the Winner" technique suggested by Dr. Marvin Zelen and a sequential Bayesian technique suggested by Dr. E. A. Gehan.

11. Stochastic Processes in Bacteriophage Growth. (J. J. Gart) More realistic stochastic models for the distribution of the burst-sizes of chemical induced mutated bacteriophages have been derived. The models are presently being fitted to available data.

Significance to the Program of the Institute

The interplay between mathematical theory, data analysis and experimental research is an important element in biomedical research. Many of the "Major Findings" reported above are new statistical techniques which may be directly applied to data collected by the medical researchers at NCI or other workers on problems in cancer research. Others are mathematical models which may elucidate the biological phenomena involved in cancer research and which may also aid in the planning of subsequent experiments or epidemiologic studies. A very few of these topics may have no specific relationship to cancer research, but are useful contributions to scientific research generally. The opportunity for initiating fundamental research on mathematics and mathematical statistics is essential for enabling members of the section to achieve professional recognition among their peers in their own scientific disciplines. More importantly, the possibility of doing such unconstrained research is a prerequisite for the consulting work of the section to be carried out at the highest professional level.

Proposed Course of the Project:

Many of the projects given in Major Findings will be continuing. In addition, Dr. Howard Shapiro shall be working on a biological system which would enable experimental confirmation of a theory of virogenesis by cancer. Dr. John J. Gart will work on a goodness of fit test for censored exponential distributions which may prove useful in analyzing certain survival curve data.

Honors and Awards

Dr. John J. Gart has served as a referee for Biometrics, The Journal of the American Statistical Association, and The Journal of Theoretical Biology. He was also an invited lecturer at Johns Hopkins University.

Donald G. Thomas and Dr. E. A. Gehan presented a paper entitled "On the performance of some two-sample tests in small samples with and without censoring" at the national meeting of the Biometric Society in Washington, D. C. in December 1967.

Dr. Howard M. Shapiro presented an invited paper "Input-output models of biological systems" at the Sixth Annual Symposium on Biomathematics and Computer Science in the Life Sciences in Houston, Texas in March 1968.

Publications

Gart, J. J. and Zweifel, J. R.: On the bias of various estimators of the logit and its variance with application to quantal bioassay. Biometrika 54: 181-187, 1967.

Gart, J. J. and Weiss, G. H.: Graphically oriented tests for host variability in dilution experiments. Biometrics 23: 269-284, 1967.

Gart, J. J.: A simple nearly efficient alternative to the simple sib method in the complete ascertainment case. Ann. Hum. Genet. Lond. 31: 283-291, 1968.

Gart, J. J.: The mathematical analysis of an epidemic with two kinds of susceptibles. Biometrics 24: (in press) 1968.

Pettigrew, H. M. and Weiss, G. H.: Epidemics with carriers: the large population approximation. J. Appl. Prob. 4: 257-263, 1967.

Pettigrew, H. M. and Mohler, W. C.: A rapid test for the Poisson distribution using the range. Biometrics 23: 685-692, 1967.

Serial No. NCI- 4268
1. Biometry Branch, OASDD, Etiology
2. Special Cancer Survey Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Third National Cancer Survey

Previous Serial Number: None

Principal Investigator: John C. Bailar, III

Other Investigators: John L. Young, Jr., Susan S. Devesa, Theodore Weiss

Cooperating Units: University of Alabama School of Medicine

Man Years:
Total: 4.0
Professional: 3.0
Other: 1.0

Project Description

Objectives:

To prepare a complete register of all new cancer cases diagnosed in the resident population of twelve defined areas during 1969-1971, plus all other cancer cases seen by doctors or hospitals in those areas; to obtain demographic data and a description of the tumor for each patient, and to study certain economic, social, and other aspects of the disease in a sample of patients.

Intensive cancer morbidity and mortality studies over a three year period in this large population will lead to the first comprehensive picture of cancer in the United States in over 20 years. The economic and social investigations have never been attempted before in this way or on this scale. They will provide the first reliable data on the real impact of cancer on a representative sample of patients, on their families, on their communities, and on the nation.

Complete coverage of population groups included in the new survey will also provide physicians and public health officials with reliable answers to important questions, such as variations in the incidence and prevalence of specific forms of the disease and the economic cost of cancer to a community. Physicians and hospital administrators in each area will also be able to plan treatment and care facilities more effectively.

Methods Employed:

In each study area the National Cancer Institute will contract with local organizations to hire field staff and gather data. Data will be requested from doctors and obtained from hospital records, death certificates, and, upon approval of their doctors, interviews with a sample of cancer patients. Local groups will also do the preliminary editing and coding of data, and will be responsible for disposition of the original records at the close of the survey. Institute staff in Bethesda, Maryland, will complete the editing and coding of data and will convert it to punched cards or magnetic tape for use in preparing detailed reports through electronic computers.

The new survey will collect basic information on every new cancer case during the 3-year period. This will include demographic data, age, place and date of birth, sex, and marital status. Medical information sought will include the primary site and histologic type of tumor, dates, locations, and methods of diagnosis and treatments. Information available from any existing cancer register or continuing survey in a study area will be utilized to avoid duplication. All reports will, of course, be treated as strictly confidential.

Doctors will be provided brief forms to be filled out for each new cancer case and forwarded to the local survey staff. Death certificates mentioning cancer will be obtained from the appropriate vital statistics office. Local survey staff or hospital personnel will abstract information on cancer patients from hospital records. There will be no actual examination of patients.

Trained interviewers, having first received written permission from attending doctors, will obtain from a selected number of patients information on such matters as the amount of disability incurred, time lost from productive activity, cost of treatment, and sources of payment. Questions will also be asked regarding religion, number of children, and socio-economic status. Some estimates will be made of the time lapse between initial symptoms, diagnosis, and treatment. Personal habits such as smoking will come under scrutiny.

The study is of such magnitude that it is necessary to do a pretest. Birmingham, Alabama, has been chosen for a 3-month preliminary study in the spring of 1968. During this time, questionnaires and procedures will be tested, cost of the main survey will be closely estimated, and usefulness of the ways the data are being obtained will be determined.

The main product of this survey will be a detailed analysis of cancer incidence rates. There will also be several ad hoc reports on special problems, such as the relation between occupation and cancer incidence, and the burden of cancer on the national economy. Summary reports on each of the study areas will be prepared for the use of local physicians, hospitals, public health workers, and other interested persons.

The table on page 609 shows the areas that we hope to include in the survey, their projected populations (which come to over 10% of the total population of the United States), and the number of new cancer cases expected to occur among residents of the survey areas during 1969-1971. There may be an equal number of old cases returning for further treatment or care, plus non-residents using medical facilities in the survey areas.

Major Findings:

None yet available.

Significance of the Program to the Institute:

Registration of all cancer cases in a defined population is the only way to acquire reliable data on cancer incidence rates. Such data have countless uses once they are collected; of particular interest to NCI will be detailed studies of the relation between cancer incidence and possible etiologic factors. These include occupation, residence history, place of birth, and many other items included in the survey. Other products of the survey that will be important to the mission and program of NCI will be studies on the delivery of medical care to a large and representative sample of cancer patients, delays in diagnosis and treatment, the direct and indirect costs of cancer (especially as related to type of cancer, stage at diagnosis, family income, and geographic area), and the extent and duration of disability from cancer.

Proposed Course of the Project:

During the first half of FY 69 we will continue laying detailed plans for the survey, making all necessary contacts in the survey areas, and training our own staff and the various field supervisors. After the opening date of the survey, January 1, 1969, the staff of the Special Cancer Surveys Section will be almost entirely concerned with supervising the operation of the program in all its aspects. Coordination of the 12 field offices with the administrative office in this section and with the data processing operations elsewhere in Bethesda will be a major undertaking, and will involve very extensive travel on our part.

Area	Counties Included	Projected 1970 Population	Estimated New Cancer Cases During 1969-1971
Atlanta (SMSA)	DeKalb, Fulton, Clayton, Cobb Gwinnett	1,415,000	14,000
Birmingham (SMSA)	Jefferson, Shelby, Walker	749,000	7,500
Dallas-Fort Worth (SMSA)	Dallas, Collin, Denton, Ellis, Tarrant, Johnson, Kaufman, Rockwall	2,209,000	22,000
Denver (SMSA)	Denver, Adams, Arapahoe, Boulder, Jefferson	1,217,000	12,000
Detroit (city)	Wayne	2,716,000	27,000
Iowa (state)	All	2,757,000	27,500
Minneapolis-St. Paul (SMSA)	Hennepin, Ramsey, Anoka, Dakota, Washington	1,742,000	17,500
New Orleans (SMSA)	Orleans, Jefferson, St. Bernard, St. Tammany (Parishes)	1,147,000	11,500
Philadelphia (city)	Philadelphia	2,102,000	21,000
Pittsburg (SMSA)	Alleghany, Beaver, Washington, Westmoreland	2,339,000	23,500
San Francisco-Oakland (SMSA)	Alameda, San Francisco, Contra Costa, Marin, San Mateo	3,188,000	32,000
Utah (state)	All	995,000	10,000
Total - 12 Survey Areas		22,576,000	225,500

SMSA=Standard Metropolitan Statistical Area

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1968

Demography Section

VETERANS ADMINISTRATION HOSPITAL (Minneapolis) (FS-22)

Title: Incidence of Prostatic and Bladder Cancer in a VA Hospital

Contractor's Project Director: Dr. George T. Mellinger

Project Officer (NCI): Dr. John C. Bailar, III

Objectives: This contract is largely service or data procurement--as a by-product of an elaborate controlled clinical trial. A large number of VA hospitals have been brought together to evaluate the effectiveness of various treatments in cancer of the prostate. For a relatively small additional cost, epidemiologic information has been gathered on these patients and in an appropriate group of controls.

Major Findings: Data are still being collected, and no analysis has been attempted except to insure that the data are of good quality.

Significance to biomedical Research and the Program of the Institute: The Biometry Branch has published a very extensive review of cancer of the prostate, and this experience has been quite useful in developing a program needed for a similar study of bladder cancer. In the past, attempts at combining a clinical trial with an etiologic survey have often been unsuccessful, but by the combination of several talents from within its own resources the Branch seems to be moving toward an economic and scientifically sound joint study.

Proposed Course: Data collection should be finished about the end of 1968, with a final report several months later.

Date Contract Initiated: 7-1-63

Current Contract Level: July 1, 1967 - June 30, 1968: $12,500

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1968

Special Cancer Survey Section

UNIVERSITY OF ALABAMA MEDICAL CENTER (Birmingham) (68-715)

Title: Third National Cancer Survey - Pretest

Contractor's Project Director: Dr. Peter Peacock

Project Officer (NCI): Dr. John C. Bailar, III

Objectives: This contract is to pretest most of the phases of data collection and processing in the forthcoming Third National Cancer Survey (see Individual Project Report NCI-). This includes:

1. Test adequacy of the forms and questionnaires: ease of using forms; efficiency in processing; clarity; acceptance by patients, etc.; codes for pre-coded items.
2. Try out administrative procedures for utility and efficiency.
3. Try out matching methods.
4. Estimate non-response rates.
5. Try alternative methods of approaching the MDs.
6. Estimate time and cost of various phases.
7. Estimate the variability among interviewers.

Major Findings: No data have yet been collected.

Significance to Biomedical Research and the Program of the Institute: See NCI- .

Proposed Course: Data will be collected for cancer cases seen in Birmingham, Alabama during the three months May-July, 1968. The data will be processed and analyzed as soon as possible after that time, so that changes can be made before the main survey opens January 1, 1969.

Date Contract Initiated: 4-19-68

Current Contract Level: July 1, 1967 - June 30, 1968: $27,300

CONTRACT NARRATIVES
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1968

Office of the Chief

HEALTH INSURANCE PLAN OF GREATER NEW YORK (63-49)

Title: Evaluation of Periodic Breast Cancer Screening by Mammography

Contractor's Project Director: Mr. Sam Shapiro

Project Officer (NCI): Mr. William Haenszel

Objectives: A. To determine whether a simple screening technique (x-ray mammography) can diagnose breast cancer early, and

B. To determine if the very early diagnosis of breast cancer is really useful in improving long term survival.

Methods Employed: This is a combination statistical-epidemiological-medical study in which a study population receiving mammography at annual intervals and a control group of 30,000 women each are being routinely followed for breast cancer experience through review of death certificates and through internal health insurance plan reports. Details of the techniques are given in a paper describing the methodology and initial findings of this study entitled, "Evaluation of Periodic Breast Cancer Screening with Mammography" by Sam Shapiro, Philip Strax, and Louis Venet, J.A.M.A. 195: 731-738, Feb. 28, 1966.

Major Findings: Preliminary findings indicate that mammography may be a potentially-useful technique in the early diagnosis of breast cancer. More details are given in two recent papers: Shapiro, Sam; Strax, Philip; and Venet, Louis: Periodic Breast Cancer Screening - The first two years of screening. Arch Environ Health, 15: 547-553, Nov. 1967; Strax, Philip; Venet, Louis; Shapiro, Sam; and Gross, Stanley: Mammography and Clinical Examination in Mass Screening for Cancer of the Breast. Cancer 20: 2184-2188, Dec. 1967.

Significance to NCI Program & Bio-Medical Research: Early diagnostic procedures may be of use in the early treatment and potential cure of cancer patients. If it develops that mammography is such a technique, it then could be extended to other populations and would then have the effect of reducing the mortality from breast cancer within the United States.

Proposed Course of Project: The project will continue as in the present manner with a large number of women being randomly assigned to mammography and another group being used as controls. These women will be followed for as long a time period as necessary to demonstrate whether the technique has any usefulness as an early diagnostic device or whether its usefulness is limited. The duration of the study is contingent upon the answers to the questions implied by the objectives.

Date Contract Initiated: November 1962

Current Annual Level: $462,550

NORWEGIAN PUBLIC HEALTH SERVICE (64-499)
UNIVERSITY OF MINNESOTA (66-919)

Title: Incidence, Prevalence and Mortality from Cancer in Selected Migrant Populations (Norwegian)

Contractor's Project Director: Dr. Einar Pedersen (64-499)
Dr. Leonard Schuman (66-919)

Project Officer (NCI): Mr. William Haenszel

Objectives: To determine the reason for difference in cancer incidence and mortality in sedente populations and migrant populations of the same ethnic and genetic composition.

Methods Employed: Standard epidemiologic-demographic methods including prospective observations on defined cohorts and case-control studies.

Major Findings: None to report as yet.

Significance to NCI Program & Bio-Medical Research: This is one of a group of migrant studies to determine those elements in the incidence and mortality from cancer which may be related to environment and those which may be related to genetic factors. Isolation of environmental elements may be useful in cancer prevention within the United States.

Proposed Course of Project: The Norwegian Public Health Service is responsible for the conduct of all phases of data collection in Norway carried out in connection with the Norwegian-British Migrant Study concerned with cancer and cardiorespiratory diseases. Symptom and demographic data have been collected for a representative sample of the Norwegian population and from brothers and sisters of migrants to the United States included

NORWEGIAN PUBLIC HEALTH SERVICE (64-499)(continued)
UNIVERSITY OF MINNESOTA (66-919)(continued)

in the U.S. study samples. The Norwegian cohorts will be followed for cancer incidence and mortality data. A companion program of case-control interviews for gastrointestinal cancer is underway in Norway and will be extended to other sites at a later date.

The University of Minnesota has defined a cohort of approximately 20,000 individuals in an insurance company that has a high proportion of policy holders of Norwegian descent and will monitor the group prospectively for mortality data. The contractor is also carrying out case-control interviews for gastrointestinal cancer among persons of Norwegian descent in Minnesota and is prepared to extend field work to other sites at a later date. All phases of the cohort and case-control studies in Minnesota are closely coordinated with ongoing work in Norway. One investigator from the Cancer Registry of Norway is in residence almost continuously at the University of Minnesota to assure close liaison.

Date Contract Initiated:	March	1964 (64-499)
	June	1966 (66-919)

Current Annual Level:	$50,000 (64-499)
	$71,500 (66-919)

TOHOKU UNIVERSITY SCHOOL OF MEDICINE (64-10)
UNIVERSITY OF HAWAII (63-558)
CALIFORNIA DEPARTMENT OF PUBLIC HEALTH (64-873)

Title: Study of Cancer Among Japanese Migrants

Contractor's Project Director: Professor Mitsuo Segi (64-10)
Dr. Richard K. C. Lee (63-558)
Dr. John E. Dunn, Jr. (64-873)

Project Officer (NCI): Mr. William Haenszel

Objectives: The purpose of this contract is to collect data bearing on the reasons for the differences in cancer incidence between Japanese on the home islands and the Japanese migrant populations in Hawaii and California. The purpose is to sort out those aspects of common cancers which may be genetically involved and those which may derive from aspects of the environment or some mixture of the two.

Methods Employed: Standard demographic techniques are employed, basically using a system of diagnosed cases and matched controls. In

TOHOKU UNIVERSITY SCHOOL OF MEDICINE (64-10)(continued)
UNIVERSITY OF HAWAII (63-588)(continued)
CALIFORNIA DEPARTMENT OF PUBLIC HEALTH (64-873) (continued)

addition there is a small population sample being matched for certain other basic information. The matched case-control technique is particularly useful in this kind of study.

Major Findings: In view of the differences in incidence of cancer of certain sites, i.e. stomach cancer, lung cancer, breast cancer between the Japanese populations on the home islands and the Japanese migrants to Hawaii and to California, it is of considerable consequence to determine whether these differences can be accounted for by environmental factors. To date, the potential information exists tending to incriminate changes in dietary habits and in smoking habits for the changes in stomach cancer, and in lung cancer in the Japanese populations.

Significance to NCI Program & Bio-Medical Research: If the studies in these migrant populations elucidate the environmental factors involved in the incidence of certain forms of cancer, and indicates those that are predominatly environmental and those which are predominantly genetic, then it may be possible to apply these findings to the United States population for purposes of cancer prevention and cancer control.

Proposed Course of the Project: Interviews of household samples in Hiroshima and Miyagi prefectures, California and Hawaii have been completed. Interviews of stomach cancer patients and matched hospital controls in Japan have been completed. Current work covers case-control interviews for cancer of the esophagus, rectum, lung and leukemia. Other sites will be added, including breast, cervix and prostate as work progresses. Acquisition of pathology protocols on diagnosed cases has been coordinated with the collection of epidemiologic data. This work has been completed for stomach, and arrangements made to collect pathology protocols for colon, rectum, and esophagus. An exploratory study of the presence of occult prostatic carcinoma in elderly Japanese males has been started.

Date Contract Initiated: July 1963 (64-10)
April 1963 (63-558)
May 1964 (64-873)

Current Annual Level: $67,300 (64-10)
$43,500 (63-558)*
$39,577 (64-873)

*Pathology support to this contract provided by Kuakini Hospital under contract 65-1003 in the amount of $27,686.

UNIVERSIDAD DEL VALLE -- FACULTAD DE MEDICINA (PH43-66-907)

Title: Epidemiology-pathology studies of cancer in Colombia

Contractor's Project Director: Professor Pelayo Correa

Project Officers (NCI): Mr. William Haenszel, Dr. John Berg

Objectives: To determine site-specific cancer incidence in Cali and other cities in Colombia and to correlate the morbidity data with pathology findings.

Methods Employed: The cancer morbidity surveys in Cali will be continued with emphasis on variation by socio-economic class and place of birth. Internal migration within Colombia is of such magnitude that two-thirds of the residents of Cali were born elsewhere. Cancer morbidity surveys on a limited scale will be undertaken in other cities thought to have greatly different site-specific incidence, such as Cartegena and Medellin.

Studies of autopsy materials and surgical specimens will be conducted in an effort to define criteria which differentiate high-risk and low-risk populations.

In Cali the development of an organ bank of tissues from persons dying from accidents and acute diseases will be utilized for study of occult tumors and premalignant conditions.

Major Findings: A pilot trial has proved the feasibility of the organ bank concept in Cali. The medical examiner's offices have been located at the School of Medicine and procedures for collection of tissues from medical examiner cases worked out.

Preliminary observations on medical examiner and hospital autopsy materials provide some provocative leads. Application of the Lauren concept of intestinal and diffuse types of stomach cancer points to substantial differences in distribution between Cali where stomach cancer is common and in Cartegena where it is rare. The method of examination of colons used in Cali has uncovered some very early atypias which cannot be called polyps. The vital statistics for Cali have consistently described a low mortality for prostatic carcinoma in the presence of a high incidence rate. Review of the pathology materials indicates the majority of these cancers were found during transurethral resection for apparent hypertrophy and showed diffuse cancer or an early malignancy in the prostate ducts, which is quite contrary to American experience and raises a question of a different type of disease.

UNIVERSIDAD DEL VALLE--FACULTAD DE MEDICINA (PH43-66-907) (continued)

Significance to NCI Program & Bio-Medical Research: The Department of Pathology at the Universidad del Valle is strongly oriented to epidemiology and geographic pathology and its activities have no counterparts in U.S. university departments of pathology. The objective in Colombia is to produce data for comparative studies in the United States and elsewhere, with emphasis on those areas providing the greatest contrasts in risks for specific cancer sites.

Proposed Course of Project: The long-range objective is the correlation of morbidity and pathology findings and the search for etiologies. In the immediate future, collection of data on gradient on cancer risks within Colombia by socioeconomic class, place of birth and current residence, etc. will be continued. Much effort will need to be placed on refinement of pathology criteria and their test as discriminants between high- and low-risk areas. Priority in study of the pathology materials will be given to stomach, colon, prostate and thyroid.

Date Contract Initiated: June 1966

Current Annual Level: $26,540

UNIVERSITY OF PITTSBURGH (62-147)

Title: Carcinogenic Hazards in Industrial Populations (Steel)

Contractor's Project Director: Antonio Ciocco, Ph.D.

Project Officers (NCI): Frank E. Lundin, Jr., M.D. and J. William Lloyd, Sc.D.

Objectives: Detection of carcinogenic exposures in industry for the protection of workers and to more fully understand susceptibility of human subjects to chemical carcinogens.

Methods Employed: A cohort of approximately 59,000 steelworkers who were employed in the Pittsburgh area in 1953 has been followed for mortality analysis from 1953 through 1961. Five additional years of mortality experience is currently being assembled. Employees in or around 1953 of approximately 10-12 coke-oven plants are compared with matched control groups of steelworkers in widely scattered geographic areas in the U.S. and Canada with regard to mortality by cause. Records of the cohorts who left the industry prior to January 1, 1967 are being checked with the Post Office Department, the Bureau of Old Age and Survivors Insurance and other governmental agencies to determine the status of these employees as of December 31, 1966. Death certificates for employees who expired in the study period are obtained from state vital statistics divisions and are classified as to cause of death.

Major Findings: Men employed on the topside of coke ovens have a 6-fold excess of lung cancer.

Significance to NCI Program & Bio-Medical Research: The uncovering of industrial hazards will lead to safer work practices within industry and to information concerning the etiology of and susceptibility to cancer.

Proposed Course of the Project: Approximately 1 additional year will be required to complete the extended and geographically expanded portions of the study described above. Collaboration with medical directors and other officials of the steel industry has been excellent.

Current Contract Initiated: December 1961

Current Annual Level: $76,000

COMPUTER USAGE DEVELOPMENT CORPORATION (43-68-981)

Title: Third National Cancer Survey Data Processing Software Contract

Contractor's Project Director: Mr. Julio Cima

Project Officer (NCI): Mr. Theodore Weiss

Objectives: A. To design and implement an efficient, economically operable, well-designed, well-documented and easily administered computer-based data processing system for the collection, transcription conversion, control and tabulation of confidential cancer survey data.

B. The system should be designed to operate for the survey's pretest and have the capability of being employed for the main survey with a minimum of modification.

Methods Employed: A project team of analyst/programmer specialists will be assigned to the project under the direction of a project leader. The project will be divided into six phases: (1) systems analysis, (2) systems design, (3) computer programming, (4) operating manual, (5) training and implementation, and (6) a management report. Faced with a critical timing problem, the contractor is required to overlap phases of the project and to implement each phase as it is completed.

Significance to NCI Program: Effective use of advanced computer technology will provide the program area with desired systems results in a time frame of weeks instead of years.

Proposed Course of Project: The contract will be completed August 16, 1968. Following the processing of pretest data, and an analysis of the effectiveness of survey forms, procedures, and the system itself, the ADP Management Staff will modify the system for use in the main survey commencing January 1, 1969.

Date Contract Initiated: April 16, 1968

Current Level: $60,000

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1968

END RESULTS SECTION

CALIFORNIA DEPARTMENT OF PUBLIC HEALTH (63-27)
UNIVERSITY OF CALIFORNIA (67-62)
CHARITY HOSPITAL OF LOUISIANA (64-83)
UNIVERSITY OF CHICAGO (65-5)
CONNECTICUT STATE DEPARTMENT OF PUBLIC HEALTH (63-1148)
EINSTEIN MEDICAL CENTER (Pennsylvania) (64-37)
INDIANA UNIVERSITY HOSPITAL (64-14)
STATE UNIVERSITY OF IOWA (64-15)
MASSACHUSETTS HEALTH RESEARCH INSTITUTE (64-13)
UNIVERSITY OF MICHIGAN (64-24)
NEW YORK MEDICAL COLLEGE (64-23)
UNIVERSITY OF VIRGINIA (67-13)

Title: End Results Evaluation

Project Officer (NCI): Dr. Sidney J. Cutler

Objectives: To obtain and analyze volume data for all cancer diagnoses, but with emphasis upon specific types of cancer and upon patients with selected characteristics, in order to study:

1. the changes over time in the use of different methods of therapy.

2. the relative effectiveness of the various treatment modalities as judged by patient survival, and any changes over time in survival.

To acquire and analyze supplementary data designed to explain further any specific finding from the relatively crude mass data.

Methods Employed: The National Cancer Institute sponsors a program in which more than 100 hospitals contribute data on the end-results of cancer treatment. The Biometry Branch has professional and technical responsibility for supervising these contracts. Information on individual patients is submitted to the Biometry Branch, according to a prescribed schedule, and in accordance with a uniform punchcard code. This program makes available to the National Cancer Institute information on a very large series of patients (e.g., 427,000 patients were treated in participating hospitals during the period 1940-1962) in a form which permits maximum flexibility in handling and analyzing the data, and also provides an extensive resource for special studies on selected series of cases. Partial support for three centralized registries (in California, Connecticut, and Massachusetts) and for nine registries in university hospitals is provided through the contract mechanism to make it possible for the participating registries to meet specified criteria regarding completeness, currency, and accuracy of information.

To obtain supplementary data, patients with pertinent characteristics are identified in the accumulated files. The medical records for these patients are reabstracted in detail using a specially designed protocol. The records of similar patients are abstracted on a prospective basis.

Major Findings: Primary emphasis during the past year has been placed on the preparation of End Results Report No. 3 to be distributed at the Sixth National Cancer Conference in September 1968. Also, on completion of a revised manual of procedures, definitions and codes.

Significance to NCI Program & Bio-Medical Research: The End Results Program provides the most comprehensive information available on major trends in cancer treatment and associated results, and provides a base for a variety of special studies.

Proposed Course of the Project: Increased attention will be given to the preparation of a series of interpretive papers for publication in a variety of scientific journals. These papers will be designed to bring the information generated by the End Results Program to the attention of various medical specialists and general practitioners.

CALIFORNIA DEPARTMENT OF PUBLIC HEALTH (63-27)
Etc. (continued)

	Date Contract Initi- ated	Current Annual Level
CALIFORNIA DEPARTMENT OF PUBLIC HEALTH (63-27)	7-1-62	$79,542
UNIVERSITY OF CALIFORNIA (67-62)	11-1-66	33,912
CHARITY HOSPITAL OF LOUISIANA (64-83)	10-1-63	33,241
UNIVERSITY OF CHICAGO (65-5)	7-1-64	19,956
CONNECTICUT STATE DEPARTMENT OF PUBLIC HEALTH (63-1148)	6-1-63	86,419
EINSTEIN MEDICAL CENTER (Pa.) (64-37)	8-1-63	10,845
INDIANA UNIVERSITY HOSPITAL (64-14)	7-1-63	34,500
STATE UNIVERSITY OF IOWA (64-15)	7-1-63	15,000
MASSACHUSETTS HEALTH RESEARCH INSTITUTE (64-13)	7-1-63	53,656
UNIVERSITY OF MICHIGAN (66-24)	9-1-65	24,480
NEW YORK MEDICAL COLLEGE (66-23)	7-1-65	16,000
UNIVERSITY OF VIRGINIA (67-13)	7-1-66	26,290

SUMMARY REPORT
EPIDEMIOLOGY BRANCH
JULY 1, 1967 - JUNE 30, 1968

The objective of the Epidemiology Branch is to test ideas concerning the origins of cancer in man through epidemiologic studies based on medical knowledge and experience and on an awareness of resources only available or best available at the national or international level. An idea is developed from existing knowledge, which may come from the laboratory, the clinic, or other epidemiologic studies. Emphasis is placed on epidemiologic research based on important new information from laboratory investigations at the National Cancer Institute and elsewhere.

Staff Changes: Andrew G. Glass, M.D. and Frederick P. Li, M.D., whose interests are in pediatric oncology and in occupational cancers, respectively, joined the staff on July 1, 1967. Wladimir Wertelecki, M.D., a pediatrician-cytogeneticist who has been working at Harvard on a computer-taken family history, joined the staff on May 8, 1968. Frank E. Lundin, Jr., M.D., Dr. P.H., transferred to the Environmental Health Sciences Division on July 15, 1967.

Outside Training: Joseph K. Wagoner, M.S., continued his training toward a doctoral degree in biostatistics at the Harvard School of Public Health: James A. Peters, D.V.M., was awarded an MPH degree at the University of Michigan. C. Richard Stark, M.D., Dr. P.H., undertook a year of clinical training in pediatrics at Georgetown University Hospital.

Research Program:

Childhood Cancer: In 1962 the Branch began to examine the relationship between cancer and congenital defects. This epidemiologic approach, little used previously, seeks an excessive coexistence of the two diseases in persons, families or communities. When diseases are found to occur together more often than can be expected by chance, each can be studied in the light of what is known of the other for clues to etiology. In the past year progress has continued to be made in these investigations.

A registry has been established of death certificates for all children who died of cancer in the United States since 1960 (total = 21,959 through 1964). These data have revealed that 1) among about 7,000 Maryland children immunized against measles in 1961 with a vaccine containing viruses capable of inducing cancer in lower species, none of the vaccinated children had died of cancer through 1964; 2) a Boston study, disputed in Great Britain, was correct in concluding that when an identical twin develops leukemia under 6 years of age, there is a high probability (1 in 5) that the co-twin will develop the disease soon thereafter; 3) brain tumors and infantile histiocytosis each aggregate among siblings to a greater extent than was previously known; 4) neuroblastoma, a cancer of nerve tissue in young children, originates largely in utero and varies over time in certain subdivisions of the U. S. in a fashion which suggests an environmental influence on its occurrence; and 5) certain cancers occur excessively in children with specific congenital defects, clarifying or suggesting associations not previously known.

Other reports published or accepted for publication during the year concerned persons at exceptionally high risk of leukemia (common to all was a distinctive genetic or cytogenetic feature); the epidemiologic and personal characteristics of children with primary liver cancer -- which appears to originate _in utero_ and is associated with certain congenital anomalies; five new cases and a review of the literature of Wilms' tumor with congenital aniridia; an analytic review of the relationship between childhood cancer and congenital defects; the sharp peak in mortality under 3 years of age in malignant histiocytosis or Letterer-Siwe disease, suggestive of a prenatal origin of the disease, and the substantial tendency of such deaths to cluster in families (5 sib-pairs, including one set of like-sex twins among 270 deaths); the lack of excessive time-space clustering of leukemia or other childhood cancer in Los Angeles County; and an analysis of the "spread" of leukemia which revealed no evidence of horizontal or vertical transmission of the disease even among persons whose contact with it was heavy. In addition, the epidemiologic characteristics of leukemia were shown to be very different from those of kuru, a disease believed to be due to one of the slowest of slow viruses.

A small contract continues with Case-Western Reserve University for follow-up of about 700 children who as newborns received oral polio-vaccine containing SV-40. The objective is to determine if cancer has been induced by this contaminant which under certain circumstances is oncogenic in lower species. To date there have been 11 deaths, none due to cancer.

Currently under study are neonatal deaths from congenital cancer, of which there were 130 in the U. S., 1960-64. As compared with type-specific cancer deaths under 5 years of age, there was among the congenital neoplasms a disproportionate number of neuroblastomas, primary liver cancer and malignant teratomas. Also under study are the national mortality of bone tumors, gonadal neoplasia, Hodgkin's disease and rhabdomyosarcoma. Because these neoplasms are rare, they have received little attention epidemiologically, and indeed cannot be so studied except in very large countries with death certificates of good quality. In contrast to extensive epidemiologic research into adult neoplasia, childhood tumors other than leukemia have largely been ignored, though such study can have important implications with respect to cancers in other age groups.

Adult Cancer, Not Occupational: Under contract with NAS-NRC a multi-faceted study of viral and other factors in the etiology of human cancer is in its third and final year. Using the prospective approach, certain diagnoses, such as infectious mononucleosis or hepatitis, among soldiers in World War II are being studied for their relationship to subsequent cancer experience. The diagnoses have been obtained from the files in the Office of the Surgeon General of the Army and current health status is determined from the Master Index of the Veterans Administration. Cases are compared with a matched sample of controls. For those who have died, death certificates are easily available through the V. A.. In the retrospective component of the study, about 3,000 Army veterans dying of cancer have been identified from V. A. death certificates, and through data abstracted from their military service record, a relationship is being sought between cancer and such events as yellow-fever vaccination or blood transfusion. The file concerning diagnoses made in Army hospitals during World War II is in danger of being destroyed because the punch-cards are old and have outlived their administrative purpose. A contract has, therefore, been provided to protect this rich epidemiologic resource by transferring the data from the punch cards to computer tape.

Another study involved follow-up of persons with bone marrow depression induced by chloramphenicol or phenylbutazone, two drugs suspected to be leukemogenic. About 150 subjects with such bone marrow depression were ascertained, principally from the Adverse Drug Registries of the AMA and FDA. There were 6 who developed leukemia; in all but 1 or 2 instances of chloramphenicol-associated leukemia the onset of the neoplasm was so soon after drug administration as to suggest that the therapy had been given for early symptoms of clinically unrecognized leukemia; i.e., that the neoplasm caused the drugs to be given, rather than the converse.

Several studies are being made of the risk of cancer among patients, or their family members, with non-neoplastic diseases characterized by defects or immunity. No excess of cancer deaths was observed in a follow-up study of 854 patients with leprosy, a disease associated with a prolonged impairment of delayed hypersensitivity. Complete family histories have been obtained of 190 patients with systemic lupus erythematosus, a primary connective tissue disorder with a variety of autoimmune antibodies. They will be used to determine whether leukemia and/or lymphoma aggregate excessively in these families. Also, the possibility that maternal autoimmune disease may induce chromosomal aberrations in the progency is being explored, using these family histories. We also reported during the year a patient with Hodgkin's disease and panaortitis, disorders which may share a relationship to immunologic abnormality, with the aortitis perhaps the consequence of increased sensitivity to radiation therapy.

Possible etiologic implications of family histories of cancer patients were explored by our staff members who made ward rounds regularly at the Clinical Center. During the past year the occurrence of multiple familial polyposis and extra-alimentary sarcomas of various types was delineated, representing possibly a variant of Gardner's syndrome (multiple familial polyposis and benign mesenchymal tumors). On the wards, the presence of several pregnant women with Hodgkin's disease has led to a study, now in progress, of the outcome of pregnancy and the health of the child in relation to the maternal disease or treatment.

Nearing completion is a study of mortality among 32,000 Roman Catholic nuns, 1900-1954, to determine the influence of this mode of life on cancer, among other causes of death.

Under contract with the Epidemiology Department of the Harvard School of Public Health, study is being made of the frequency of cancers of various sites among women treated with radiation 15 to 35 years ago for benign gynecological abnormalities.

One staff member, through the application of numerical taxonomy is clarifying complex tabulations of measurement data through the creation of diagrammatic representations. The procedure has been applied to strains of influenza A and B virus and produced groupings which suggest a basis, perhaps more systematic than previously, for selection of strains to be used in vaccine manufacture. The technique applies as well to other entities, from molecular biology and cytogenetics to population studies. Possibly, it may provide a means for predicting oncogenicity of new virus strains by depicting their relationship to clusters of strains of known oncogenicity.

Investigators at the University of Pennsylvania are attempting to develop a computer for mass-screening of human chromosomes under a contract initiated by the Collaborative Research Branch of NCI and transferred for administrative reasons two years ago to the Epidemiology Branch. Although the outlook remains promising, grant support is now deemed more appropriate. Hence, the contract will be terminated in FY-69.

The Hagerstown (Md.) field station will be discontinued in June 1968. It was established about a decade ago to study cancer occurrence in relation to small variations in environmental circumstances through Washington County. The resource developed, of greater value as a general population laboratory than for cancer specifically, will continue to be used by the Epidemiology Department of The Johns Hopkins School of Hygiene and Public Health for research particularly by its graduate students.

Occupational Cancer: Nearing completion is a study of causes of death among 8,047 smelter workers. The results reveal an excess of lung cancer apparently related primarily to arsenic exposure.

Under contract with Hiroshima University substantial progress has been made in delineating a marked excess of oral and respiratory-tract cancers among former workers engaged in the manufacture of mustard gas near Hiroshima. An interim report of results to date should be ready by June 1968.

Under analysis are 4,000 deaths among members of the American Chemical Society, 1948-1967. The purpose is to seek new clues to chemical carcinogenesis in man.

A great handicap in the epidemiologic study of occupational cancers is the immensity of each study if a simple record-linkage procedure is not available. Tracing workers to determine their survival status over several decades usually involves one professional staff member, a half-dozen clerks, 3-5 years and substantial funds. The increment of new information thus derived may be small. A great asset in such studies would be the establishment of a National Death Index which would list each death in the U. S. by name, place and social security number. Linkage to past employment records would then be simple, and occupational studies could be made in profusion. The establishment of the Index is being considered by the Environmental Health Sciences Division of NIH.

Veterinary Cancer: In 1961 the Epizootiology Section was initiated in the expectation that research into neoplasia among domestic animals would provide new insight into the origins of human cancer. There was, then, an absence of systematically collected data from epidemiologic research. The Section quickly developed a system for making standard observations on a mass-scale and to permit machine-processing and retrieval. The system was widely adopted both here and abroad. Virtually all U. S. veterinary medical colleges use it and send abstracts from each hospital or clinic admission record to our field station at Michigan State University for data processing. Each contributing institution receives a monthly listing of its data; NCI has the pooled data for its use. The effort to date has largely been in the establishment and popularization of the system. During the year a report has been published concerning the frequency of lymphoma in the Boxer breed, which is about 6 times greater than in other purebred dogs.

The resource can be used to identify animals with specific diseases -- not only cancer but any ailment -- for experimental therapy or for etiologic studies involving manipulation of breeding or of the environment in relation to effects on the offspring. Emphasis during the coming year will be on the analysis of the data in a collaborative effort with physicians in the Branch. Though it is time for this arrangement in any event, the planned shift of the resource to our Bethesda offices is partially the consequence of the expected departure of the Head of the Section was has accepted a position at the Mayo Clinic. The field station will be closed, and one senior veterinarian will transfer to Bethesda from East Lansing to continue the system and to work with us in the analysis of the data.

Serial No. NCI-4325
1. Epidemiology Branch, OASDD,
   Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Planning and Development in Cancer Epidemiology

Previous Serial Number: Same

Principal Investigator: Robert W. Miller, M.D.

Other Investigators: J. F. Fraumeni, Jr., M.D., R. A. Tjalma, D.V.M., and other professional staff of the Epidemiology Branch

Cooperating Units: None

Man Years:
Total: 2.0
Professional: 2.0
Other: 0.0

Project Description

Objectives:

1. To originate new epidemiologic approaches to the study of cancer causation in man and animals.
2. To develop sources of data related to specific epidemiologic problems.
3. To stimulate epidemiologic research in other health agencies.
4. To develop an on-the-job training program for medical and statistical officers.

Methods Employed:

The Program being planned is based on:

1. Leads from animal experimentation.
2. Prospective studies (in retrospect) which relate cancer occurrence to events recorded prior to the onset of cancer in medical examinations obtained in a standard fashion from large numbers of persons (examples -

clinical health-surveys, business executive annual health examinations, and military medical examinations).

3. Retrospective studies based on questionnaires obtained by personal interview or by mail, for a comparison of persons with a specific type of cancer and a similar group without this cancer.

4. Prospective intramural studies which involve the establishment of a clinical or other screening program where none has existed before, as for example, in a rural area or among groups of persons believed to be at a high risk of cancer.

As specific epidemiologic questions arise from laboratory or clinical observations, sources of field data are developed to answer these. Conversely, the Branch seeks by its surveys to raise questions which can be answered by laboratory or clinical studies. Parallel with this, to fill the tremendous deficiency in qualified personnel, the selection and training of young physicians, veterinarians, and statisticians must take place. The principles they learn from the guided planning, execution, and analysis of a program in cancer epidemiology are applicable in full to studies of the genesis of congenital malformations, heart disease, arthritis, mental aberrations, or the effects of ionizing radiation.

Significance to Bio-Medical Research and the Program of the Institute:

If the example set by the Branch is a good one, interest will be stimulated among medical scientists in the use of epidemiologic methods for their research. In consequence there may develop a further recognition of the usefulness of office and hospital records for survey studies. With this would go an appreciation for the need to adapt the standard medical records for epidemiologic research. These developments, in turn, could promote an interest in looking beyond the walls of hospitals and medical centers for opportunities in medical research.

Proposed Course of Project:

To develop opportunities in cancer epidemiology as they arise.

Honors and Awards

Robert W. Miller, M.D.

Speaker

"Radiation and Other Factors in the Etiology of Leukemia" - Radiological Health Division, PHS, Rockville, Md., July 5, 1967.

"Relationship between Cancer and Congenital Defects" - Clinical Conference on Neoplasia in Childhood, M.D. Anderson Hospital, Texas, November 9, 1967; Medical College of Virginia, Richmond, Va., January 9, 1968;

Children's Hospital, Cincinnati, Ohio, February 26, 1968; Annual Meeting of Special Virus-Leukemia Program, Airlie House, Warrenton, Virginia, March 11, 1968; and Tokyo Pediatric Society, April 25, 1968.

"Monitoring Vaccines from Human Oncogenicity" - Conference on Cell Cultures for Virus Vaccine Production sponsored by DBS, NIH, November 8, 1967.

"The Viral Etiology of Human Leukemia: An Epidemiologic Evaluation" - International Conference on Leukemia-Lymphoma, University of Michigan, Ann Arbor, Michigan, October 9, 1967.

"Neuroblastoma: Epidemiologic Approach to Its Origins" - Neuroblastoma Conference, Orcas Island, Washington, September 14, 1967.

"Epidemiology of Congenital Defects" - Second International Teratology Workshop, Kyoto, Japan, April 4, 1968.

"Search for an Association of Wilms' Tumor with Specific Congenital Defects" - Second International Teratology Workshop, Kyoto, Japan, April 4, 1968.

"Persons at Exceptionally High Risk of Leukemia" - Joint Meeting of the Japanese Society for Human Genetics and the Congenital Anomalies Research Association for Japan, Tokyo, April 9, 1968.

"Syndrome Delineation and Other Uses of Epidemiology" - Medical College of Virginia, Richmond, Virginia, January 9, 1968; University of Tokyo, April 26, 1968.

"Etiology of Leukemia" - Children's Hospital, Cincinnati, Ohio, February 26, 1968.

"Childhood Cancer Deaths in Sibs" - American Pediatric Society, Atlantic City, New Jersey, May 1, 1968.

"Childhood Cancer and Congenital Defects: A Study of U.S. Death Certificates, 1960-64" - The Teratology Society, Buck Hill Falls, Pennsylvania, May 15, 1968.

Committees:

Advisory Committee for the Atomic Bomb Casualty Commission

Core Group, Long-range Biomedical and Psychosocial Effects of Nuclear War, New York Academy of Sciences.

Epidemiology Working Group, SVLP (Chairman)

Reproductive Biology Study Section, NIH

Subcommittee on Solid Tumors in Children (Parent Committee, Solid Tumor Task Force)

Program Planning Committee, Tenth International Cancer Congress

Advisory Group on Air Quality Criteria for Photochemical Oxidants, National Center for Air Pollution Control

Conference on Chronic Disease Epidemiology, Division of Environmental Health Sciences

Advisory Committee on AEC Contract with University of Pittsburgh for Study of AEC Workers.

Biometry and Epidemiology Advisory Committee of FDA

Program Planning Committee, Epidemiology Section, APHA

Rubella Vaccine Meeting, DBS

Joseph F. Fraumeni, Jr., M.D.

Speaker:

"Multiple Polyposis and Malignant Mesenchymal Tumors in a Kindred: A Genetic Variety of Hereditary Polyposis?" - The Teratology Society, 7th Annual Meeting, Estes Park, Colorado, May 25, 1967.

"Radiation and Other Factors Associated with an Increased Risk of Leukemia" - Population Studies Program, National Center for Radiological Health, PHS, Rockville, Maryland, July 5, 1967.

"Constitutional Disorders Predisposing to Leukemia-Lymphoma" - Symposium on Comparative Morphology of Hematopoietic Neoplasms, sponsored by the Armed Forces Institute of Pathology and National Cancer Institute, Washington, D.C., March 12, 1968.

"Wilms' Tumor and Congenital Aniridia" - The Teratology Society, 8th Annual Meeting, Buck Hill Falls, Pennsylvania, May 15, 1968.

"Aniridia-Wilms' Tumor Syndrome" - National Foundation - March of Dimes, First Conference on Clinical Delineation of Birth Defects, The Johns Hopkins Hospital, Baltimore, Maryland, May 20, 1968.

"Epidemiologic Aspects of Bladder Cancer" - Conference on the Epidemiology and Etiology of Human Bladder Cancer, sponsored by the New England Deaconess Hospital (Cancer Research Institute) and Boston University School of Medicine, Boston, Massachusetts, June 7, 1968.

Committee Membership:

Associate Editor, Journal of the National Cancer Institute.

Attending Physician, Clinical Center, National Cancer Institute.

New Membership:

American College of Physicians (Fellow).

Epidemiology Discussion Group, Planning Program for Chemical Carcinogenesis, National Cancer Institute.

Biometry and Epidemiology Contract Review Committee, National Cancer Institute.

PHS Committee on Uranium Miners Studies.

Teratology Society.

Society for Epidemiologic Research.

Consultant:

Evaluation Committee of the VA Cooperative Prostate Cancer Research Group.

Rochester University School of Medicine.

Publications

Fraumeni, J. F., Jr.: Bone-marrow depression induced by chloramphenicol and phenylbutazone: Leukemia and other sequelae. J.A.M.A. 201: 828-834, 1967.

Fraumeni, J. F., Jr.: Stature and malignant tumors in bone in childhood and adolescence. Cancer 20: 967-973, 1967.

Fraumeni, J. F., Jr., and Miller, R. W.: Epidemiology of human leukemia: recent observations. J. Nat. Cancer Inst. 38: 593-605, 1967.

Fraumeni, J. F., Jr., and Miller, R. W.: Leukemia mortality: downturn in rates in the United States. Science 155: 1126-1128, 1967.

Fraumeni, J. F., Jr., and Thomas, L. B.: Malignant bladder tumors in a man and his three sons. J.A.M.A. 201: 507-509, 1967.

Fraumeni, J. F., Jr., Herweg, J. C., and Kissane, J. M.: Panaortitis complicating Hodgkin's disease. Ann. Intern. Med. 67: 1242-1248, 1968.

Fraumeni, J. F., Jr., Vogel, C. L., and Easton, J. M.: Sarcomas and multiple polyposis in a kindred. A genetic variety of hereditary polyposis? Arc. Int. Med. 121: 57-61, 1968.

Fraumeni, J. F., Jr.: Constitutional diseases predisposing to leukemia and lymphoma. Nat. Cancer Inst. Monograph. In Press.

Mantel, N., and Stark, C. R.: Paternal age in Down's syndrome. Amer. J. Mental Def. 71: 1025, 1967.

Miller, R. W.: Childhood cancer mortality in U.S.A. and Japan. Tohoku J. Exper. Med. 91: 103-107, 1967.

Miller, R. W.: Prenatal origins of mental retardation: epidemiologic approach. J. Pediat. 71: 455-458, 1967.

Miller, R. W., and Fraumeni, J. F., Jr.: "Leukemia Houses." Ann. Intern. Med. 67: 674-676, 1967.

Miller, R. W.: Persons at exceptionally high risk of leukemia. Cancer Res. 27 Part 1: 2420-2423, 1967.

Miller, R. W.: Effects of ionizing radiation from the atomic bomb on Japanese children. Pediatrics 41: 257-263, 1968.

Miller, R. W.: Monitoring vaccines for human oncogenicity. Nat. Cancer Inst. Monograph. In Press.

Miller, R. W.: Syndrome delineation and other uses of epidemiology. Pediat. Clinics of N. America. In Press (May 1968).

Miller, R. W.: The viral etiology of leukemia: an epidemiologic evaluation. Proceedings of the International Conference on Leukemia and Lymphoma. Lea & Febiger, 1968. In Press.

Stark, C. R., and Mantel, N.: Lack of seasonal - or temporal-spatial clustering of Down's syndrome births in Michigan. Amer. J. Epid. 86: 199-213, 1967.

Serial No. NCI-4325

Epizootiology Section
July 1, 1967 - June 30, 1968

(ADDENDUM)

Publications, formal presentations, conferences, awards and honors.

R. A. Tjalma, D.V.M., M.S.

1. Member, APHA Epidemiology Section Council.
2. Member, Epidemiology and Disease Control Study Section, DRG-NIH.
3. DRG Study Section site visits: California (2); Johns Hopkins University (2).
4. Presented course in epidemiology and public health, Michigan State University, March 1968.
5. Tjalma, R. A., "Implications of Animal Cancers to Human Neoplasia: Epidemiologic Considerations", Int. J. Cancer: 3, 1-6 (1968).

W. A. Priester, D.V.M., M.P.H.

1. Priester, W. A., "Canine Lymphoma: Relative Risk in the Boxer Breed", J NCI, Vol 39, #5, November 1967.
2. Priester, W. A., "Standard Nomenclature of Veterinary Diseases and Operations, the Veterinary Medical Data Program and Hematopoietic Neoplasms", presented at AFIP-NCI symposium on Comparative Morphology of Hematopoietic Neoplasms, March 11-12, 1968. To be published in "Proceedings".
3. Consultant to U. S. Agency for International Development in Veterinary Medical Data Processing. Initial assignment AID/Montevideo, Uruguay.

G. W. Robinson, D.V.M., M.P.H.

1. Robinson, G. W., "Birth Characteristics of Children with Congenital Dislocation of the Hip", Amer. J. Epidem., 87 (2), March 1968.
2. Robinson, G. W., "Characterization of Several Canine Populations by Age, Breed, and Sex", J. Amer. Vet. Med. Assn., 151 (8): 1072-1078, Oct. 15, 1967.
3. Robinson, G. W., "A Comparison of Licensed and Hospital Dog Populations", Amer. Vet. Med. Assn., 152 (8), April 15, 1968.

Serial No. NCI-4360
1. Epidemiology Branch, OASDD, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Epidemiologic Studies of Childhood Cancer

Previous Serial Number: None

Principal Investigators: Robert W. Miller, M.D., and Joseph F. Fraumeni, Jr., M.D.

Other Investigators: Miriam D. Manning, M.D., Andrew G. Glass, M.D., Frederick P. Li, M.D., and Judith A. Hill, B.A.

Cooperating Units: Children's Cancer Research Foundation, Boston, Massachusetts

Man Years:
Total: 2.5
Professional: 2.0
Other: 0.5

Project Description

Objectives:

To use existing data resources for the identification of groups of children at unusually high risk of type-specific neoplasia.

Methods Employed:

Review of hospital charts, tabulation of national vital statistics, analysis of birth and death certificates, prospective studies of children with diseases or exposures suspected to be oncogenic, and compilation of published case reports. A registry has been established of death certificates for all children who died of cancer in the U.S. since 1960 (total = 21,959 through 1964).

Major Findings:

1. A compilation of case reports of certain genetic diseases suggests that the heritable disorders associated with cytogenetic defects (mongolism, Bloom's syndrome, Fanconi's syndrome, and possibly D-trisomy and Klinefelter's syndrome) predispose to leukemia, whereas disorders with

with immunologic deficiencies (ataxia-telangiectasia, Wiskott-Aldrich syndrome, agammaglobulinemia, and possibly Chediak-Higashi syndrome) are related to the lymphomas. These observations support the notion that the preexisting abnormalities have pathogenic significance, rather than being concomitants of the neoplastic process.

2. Research from the Branch has revealed that among 28 children under age 4 years hospitalized with congenital aniridia, 7 developed Wilms' tumor in a follow-up survey, bringing to 22 the total number of cases known with this association and allowing fuller characterization of the syndrome. The extraordinary risk of Wilms' tumor among aniridic children should provide leads to the etiology of the tumor and should permit the early diagnosis and management of the tumor when it is associated with the anomaly.

3. Children with primary liver cancer were found to have an increased occurrence of congenital hemihypertrophy and extensive hemangiomas (anomalies which also occur in excess with both Wilms' tumor and adrenocortical neoplasms), as well as an unusual frequency of antecedent liver diseases (cirrhosis, hepatitis, biliary atresia, de Toni-Fanconi syndrome, and von Gierke's disease). The peak incidence of liver neoplasia in early childhood seems related to coexisting anomalies such as hemihypertrophy and to liver damage following biliary atresia or neonatal hepatitis, whereas liver disease due to nonspecific or metabolic cirrhosis is a precursor to neoplasia in older children and adults. Geographic variation in liver cancer mortality was found among children under 5 years of age, suggesting an environmental influence on the early peak.

4. Neuroblastoma mortality showed variations in time-space distribution and perhaps in sex ratio which were greater than those yet described in similar studies of other childhood cancers, suggesting an environmental influence on its occurrence. Conclusive evidence of an association with specific congenital defects could not be found in a hospital series comparable in size to those which demonstrated congenital defects with leukemia, Wilms' tumor, adrenocortical neoplasms, and liver cancers. Therefore, the peak frequency in early childhood for certain cancers, suggesting prenatal inception, does not necessarily signify a relation to congenital defects.

5. Mortality from malignant histiocytosis or Letterer-Siwe disease showed a peak occurrence in the first two years of life, with a rapid decline after age three. The finding of 5 sets of siblings dying of this disorder suggests inherited factors in its pathogenesis.

6. A study of childhood cancer deaths among sibs confirmed the high probability of leukemia among identical twins and revealed an excess frequency of brain tumors among non-twin sibs.

7. A study of neonatal cancer deaths (under 1 month of age) revealed a disproportionate number of teratomas, liver cancers, and neuroblastomas. The occurrence of congenital cancer provides evidence for the prenatal origins of certain neoplasms and reveals the extent to which these cancers may develop during intrauterine life.

Significance to Bio-medical Research and the Program of the Institute:

Epidemiologic study of cancer in childhood, which has received little attention until recently, is providing new insight into the origins of cancer in general. Particularly profitable has been the recognition of the excessive coexistence of certain cancers and congenital defects, a circumstance which accelerates understanding of the etiology of both diseases. The epidemiologic technique involved might be termed laterospective, for the investigator looks sideways in a search for concurrent diagnoses. It appears that this approach offers new opportunities for epidemiologic research not only with respect to cancer and congenital defects, but to disease in general. Furthermore, the delineation of high risk groups has therapeutic implications, enabling early recognition and therapy of the associated neoplasms.

Publications

Fraumeni, J. F., Jr., Geiser, C. F., and Manning, M. D.: Wilms' tumor and congenital hemihypertrophy: Report of 5 new cases and review of literature. Pediatrics 40: 886-899, 1967.

Fraumeni, J. F., Jr.: Constitutional diseases predisposing to leukemia and lymphoma. Nat. Cancer Institute. Monograph. In Press.

Fraumeni, J. F., Jr., and Manning, M. D.: Achondroplasia and leukaemia. Brit. Med. J. 3: 680, 1967.

Fraumeni, J. F., Jr., and Miller, R. W.: Adrenocortical neoplasms with hemihypertrophy, brain tumors, and other disorders. J. Pediat. 70: 129-138, 1967.

Fraumeni, J. F., Jr., Miller, R. W., and Hill, J. A.: Primary carcinoma of the liver in childhood: an epidemiologic study. J. Nat. Cancer Institute. In Press (May 1968).

Glass, A. G., and Miller, R. W.: U. S. mortality from Letterer-Siwe disease. Pediatrics. In Press.

Miller, R. W.: Relation between cancer and congenital defects: an epidemiologic evaluation. J. Nat. Cancer Institute. In Press (May 1968).

Miller, R. W., Fraumeni, J. F., Jr., and Hill, J. A.: Neuroblastoma: epidemiologic approach to its origins. Amer. J. Dis. Child. 115: 253-261, 1968.

Serial No. NCI-4333
1. Epidemiology Branch, OASDD, Etiology
2. Special Cancer Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Epidemiologic Studies of Cancer in Washington County, Maryland

Previous Serial Number: Same

Principal Investigator: P. Geiser, R.N., M.Sc.

Other Investigators: None

Cooperating Units: Johns Hopkins University School of Hygiene and Public Health, and Washington County and State of Maryland Health Departments

Man Years:
Total: 3.0
Professional: 2.0
Other: 1.0

Project Description

Objectives:

1. To determine epidemiologic patterns of cancer morbidity and mortality in Washington County, Maryland - a population consisting of rural and urban elements.
   a. To relate geographic patterns of cancer mortality to measurable variations in environmental factors in search of clues for the etiology of human cancer.
   b. To test and develop hypotheses concerning the etiology of specific forms of cancer by measuring the risk of the disease according to a number of characteristics including smoking histories, religion, education and marital histories.
   c. To study the temporal-spatial relationship of cancer cases and by the use of existing data to study the familial distribution of cancer.
2. Training and Research in Epidemiology to be conducted by The Johns Hopkins School of Hygiene and Public Health utilizing data collected by the Public Health Service since 1921.

Methods Employed:

Environmental Aspects:

1. Personal characteristics and environmental information for residents of the rural districts during the period 1948-1959 were obtained by house-to-house interviews, electric company records, 1950 census records and from geological, agricultural and isoradioactivity contour maps.

2. Life table analysis was used to search for relationships between the environment and various causes of death.

3. An alphabetical name listing makes this information for the population readily available for additional health research studies.

Special Health Census:

In mid-1963 a contract supported census including information of importance to the study of disease was carried out by approximately 100 enumerators under the direction of scientists at the Johns Hopkins School of Hygiene and Public Health.

Deaths:

IBM cards for all resident cancer deaths (5,000-6,000) occurring from 1900 to the present are maintained, and an alphabetical name list is available for record linkage studies.

Cancer Cases:

Records of cancer cases diagnosed since January 1, 1948 in the three hospitals utilized by Washington County residents have been located by a search of hospital files, and medical and social information has been abstracted and punched on IBM cards.

The records of all newly diagnosed cases of cancer of Washington County residents hospitalized at Washington County Hospital have been abstracted weekly for inclusion in the Washington County Cancer Registry and for our files.

The records of Washington County residents with the diagnosis of cancer in both the Western Maryland State Hospital, Hagerstown, and the Veterans Administration Hospital in Martinsburg, West Virginia, have been abstracted once a year for the same purpose.

A search of the 1967 pathology and cytology reports at the laboratory at Washington County Hospital has been made to check for cases which may have been missed in the weekly abstraction of case records.

A search of 1967 Emergency Room records has been made to detect cancer cases that had not been hospitalized. Approximately 75 new cases of skin cancer were found in this way.

All hospital abstracts (4,000-5,000 from 1948 to now) have been punched on IBM cards and file cards have been made (alphabetical by patient's name, primary site, father's name, spouse's name) and have been edited and corrected.

Cervical Cancer Study:

Cervical cancer cases hospitalized since 1948 now total 541, of which 470 have been located in the 1963 Health Census. Controls were chosen for the 470 from the census by starting to search for the case's name alternately up or down the list for the first woman whose birthday falls within the same five year age group as the case. These cases have been coded and punched and many runs made comparing attributes. In both cases and controls, there are women who have been divorced, widowed and remarried. Searches are being conducted to get as much information as possible on all the husbands.

A paper on Patterns of Response to a Cervical Cytology Screening Program has been submitted to Public Health Reports for publication.

Major Findings:

The Field Studies Hagerstown Project was initiated about a decade ago to study cancer occurrence in relation to small variations in environmental circumstances throughout Washington County. However, the resource has gradually developed to be of a greater value as a general population laboratory than for testing specific hypotheses concerning the causation of cancer.

Significance to Bio-medical Research and the Program of the Institute:

Because of the shift of emphasis in program, the Hagerstown field station will be discontinued in June 1968. It will continue to be used by the Epidemiology Department of The Johns Hopkins School of Hygiene and Public Health for research and training in epidemiology.

Proposed Course of Project:

To be terminated in June 1968.

Publications

Comstock, G. W., and Lundin, F. E., Jr.: Parental smoking and perinatal mortality. Amer. J. Obstet. Gynec. 98: 708-718, 1967.

Serial No. NCI-4358
1. Epidemiology Branch, OASDD, Etiology
2. Special Cancer Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Cancer Mortality in Smelter Workers

Previous Serial Number: Same

Principal Investigator: A. M. Lee, Ph.D.

Other Investigators: J. F. Fraumeni, Jr., M.D. and N. S. Wagoner, B.A.

Cooperating Units: Certain companies and the Salt Lake City Occupational Health Field Station, Occupational Health Program, PHS.

Man Years:
Total: 2.0
Professional: 1.5
Other: .5

Project Description

Objectives:

1. To determine whether exposure to arsenic trioxide results in increased cancer mortality and whether employees who have had extremely heavy exposures are at an especially high cancer risk.

2. To determine whether exposure to sulphur-bearing gases is associated with excessive cancer mortality.

Methods Employed:

1. Photocopies have been made of employment records of all employees of certain smelters with one or more years of employment, at least part of which was between November 1938 and December 31, 1957. Approximately 8,600 employees were involved, about 1800 of them currently employed or on pension.

2. For each employee the following information has been coded and keypunched for use on the IBM computer: name, date and place of birth, and social security number. Departments and dates of employment were also coded; and keypunching of this information was completed early in 1967.

3. For the 5200 individuals in the study who were not currently employed or who were reported dead with place and/or date of death unknown, follow-up through December 31, 1964 was carried out through the Social Security Administration's claims files, the Internal Revenue Service's tax files, and the death registration files in the States where the smelters were located. All study members have social security numbers and this has facilitated the search. Only 880 persons remained unknown (not known to be living or dead) when follow-up attempts ceased in September 1967.

4. Copies of approximately 2100 death certificates for deceased employees have been obtained from State health departments and coded as prescribed by the National Vital Statistics Division. Death information has been coded and keypunched for computer processing.

5. Currently all data cards have been edited and the mortality experience of the smelter workers is being analyzed by electronic computer. The death rates for specific causes of death for the entire study group are being compared with rates of the States in which the smelters are located. Further, comparisons are being made within the study group to determine how cancer mortality varies with degree and length of exposure to certain chemicals, particularly (1) arsenic trioxide, (2) sulfur dioxide, and (3) silica. The life-table method is used to calculate expected deaths for various causes and these are compared with the observed deaths in the study group.

Major Findings:

These will become available when the above-described data processing and analyses have been completed during 1968.

Significance to Bio-medical Research and Program of the Institute:

Since exposure to arsenical compounds is common in both industry and agriculture, and since arsenic is a general air pollutant as well as a constituent of tobacco, large numbers of people are exposed to this potential carcinogen. Therefore, it is important that the biologic effects of arsenic be elucidated.

Proposed Course of Project:

Computer processing of data for the study should be completed by the end of Fiscal Year 1968 and the results reported soon after that date.

Serial No. NCI-4375
1. Epidemiology Branch, OASDD, Etiology
2. Special Cancer Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Numerical Taxonomic Methods in Epidemiologic Research

Previous Serial Number: None

Principal Investigator: Anna M. Lee, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.25
Professional:	0.25
Other:	0.00

Project Description

Objectives:

To use the methods of numerical taxonomy in classifying groups of viruses in an attempt to predict oncogenicity, chemical structure and polyvalence required in the manufacture of vaccines.

To apply these techniques to chromosomes and other entities on which multivariate observations have been made.

Methods Employed:

Collection of data from (a) influenza virus HAI experiments, (b) adenovirus serologic experiments, (c) chromosome studies, (d) human mycoplasm metabolic inhibition experiments, (e) biochemical data from studies of the genetic code, and (f) studies of a similar nature; analysis by numerical taxonomic methods; and interpretation and reporting of results.

Major Findings:

1. The methods of numerical taxonomy have been employed in the analysis of serologic experiments involving oncogenic adenovirus strains, yielding meaningful results. From earlier investigations of the use of numerical taxonomic methods in classifying influenza virus strains, two types of

cluster analysis were found useful for the study: (a) principal components analysis and (b) Sokal and Michener's clustering by average linkage (weighted pair-group method). These same methods have been used in studying human mycoplasma strains.

2. Similarities of chromosomes, measured on a pattern recognition device called Confex by Dr. C. R. Stark and an associate, have been subjected to analysis and groups of chromosomes evolved. The results have been presented pictorially.

3. Similarities between triplets of bases (trinucleotides or codons), as measured with data from radioactive filtration experiments performed in the study of the genetic code by Dr. M. Nirenberg and associates, have been analyzed and groups of triplets evolved.

Significance to Bio-medical Research and the Program of the Institute:

A classification of influenza virus strains resulting from numerical taxonomic analysis has potential value in preparation of a vaccine; one might select a member from each group or cluster of viruses found in the analysis for inclusion in the vaccine. The analysis of oncogenic adenovirus strains may have predictive value in terms of the oncogenicity of a newly isolated virus shown to be serologically related to a previously identified group of viruses. Taxonomic studies of chromosomes can be used to evaluate pattern recognition devices designed to mechanize the reading of slides; such studies may also be useful in revealing previously unrecognized relationships between chromosomes. Analysis of data from studies of the genetic code and graphical representation of results has suggested relationships between codons (triplets of bases) not previously realized.

Proposed Course of Project:

To investigate the numerical taxonomic methods further, to continue to look for additional uses of the methods in bio-medical research, and to extend the analyses already begun.

Publications

Lee, A. M.: Numerical Taxonomy and Influenza B Virus. Nature (London), 217: 620-622, 1968.

Other Presentations

Lee, A. M.: "Numerical Taxonomy: An Approach and its implications." Invited paper given at joint meeting of Biometric Society and American Statistical Association in Washington, D.C., December 1967. Abstract appeared in Biometrics 23: 605, 1967.

Serial No. NCI 4338
1. Epidemiology Branch, OASDD, Etiology
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Cancer Mortality Among Catholic Brothers and Sisters in Several Teaching Orders in the United States

Previous Serial Number: Same

Principal Investigator: J. F. Fraumeni, Jr., M.D.

Other Investigators: J. W. Lloyd, Sc.D., and E. M. Smith, M.Sc.

Cooperating Units: Division of Environmental Health Sciences, NIH, and University of North Carolina (Institute for Research and Social Science)

Man Years:
Total: 1.9
Professional: 0.9
Other: 1.0

Project Description

Objectives:

1. To determine the mortality in some of the larger Catholic teaching orders for all causes and particularly mortality attributed to specific cancer sites.

2. To compare the risk of dying of cancer of specific sites with the corresponding risk in the general population to determine whether significant excesses or deficits exist for any cancer site.

Methods Employed:

1. Information on approximately 70,000 individuals comprising 62 Catholic religious communities from 1900 to 1954 has been gathered, coded and analyzed by means of IBM punch cards in another study carried out in the Institute for Research and Social Science of the University of North Carolina. Although the fact of death was recorded and punched, no attempt was originally made to determine cause.

2. Through a contract with the above-named institute the identifying data on all deceased individuals has been duplicated and a deck of IBM cards on the entire population under study has been furnished.

3. The causes of death have been obtained through appropriate channels from the death certificate of each deceased person.

4. Tabulation and biometric analysis is being made of the mortality data to discover similarities to and differences from similar data relative to the general population.

Major Findings:

A published paper has described the marked excess of breast-cancer mortality, stressing the occurrence of a "break" in the age curve at menopause. The phenomenon appears to have a biological basis and to be related particularly to the unmarried state. Preliminary data among nuns suggest a deficiency of uterine cancer and excesses in ovarian and digestive cancers.

Significance to Bio-medical Research and the Program of the Institute:

This study, which is much larger in scope than a similar one recently completed by Dr. Taylor, Mr. Carroll and Dr. Lloyd, should help to clarify some fragmentary evidence that the incidence of certain forms of cancer is different in some religious orders than in the general population, due to variations in the way of life or to the sexual, reproductive, or hormonal differences connected with the marital state. Pursuance to the underlying cause for differentials observed in the populations compared may advance the understanding of the genesis of cancer.

Proposed Course of Project:

Analysis of the Sisterhood data is nearly completed. A manuscript describing the cancer experience at each site is being prepared.

Serial No. NCI-4372
1. Epidemiology Branch, OASDD, Etiology
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Significance of Leukemia Clusters

Previous Serial Number: None

Principal Investigators: Andrew G. Glass, M.D., Robert W. Miller, M.D., and Joseph F. Fraumeni, Jr., M.D.

Other Investigators: Judith A. Hill, B.A.

Cooperating Units: None

Man Years:
Total: 0.4
Professional: 0.2
Other: 0.2

Project Description

Objectives:

To test the hypothesis that leukemia has an infectious etiology by evaluating the evidence that the disease aggregates in time and space.

Methods Employed:

All leukemia deaths in children in Los Angeles County during the years, 1960-64, were plotted on a large map of the area using information from death certificates. The Ederer-Myers-Mantel procedure was employed to detect abnormal clustering. Further, an attempt was made to generate leukemia clusters, and the local rates so produced were compared with the overall rates for cancer in the county.

An analytic review was made of (1) reported leukemia "outbreaks" (including leukemia "houses") and statistical studies to evaluate the tendency toward case clustering, (2) evidence for patient-pet clustering of leukemia or other "virus-like" patterns, and (3) the epidemiology of Burkitt's tumor.

Major Findings:

No significant clustering in the Los Angeles area was found by statistical clustering analysis, although local leukemia death rates could be forced to very high levels by manipulating groups of cases to produce clusters.

Review of pertinent studies revealed little evidence to support the hypothesis that aggregates of leukemia or patient-pet clusters represent foci of infection.

Significance to Bio-medical Research and the Program of the Institute:

Whereas no clustering in Los Angeles was apparent when leukemia deaths were looked at dispassionately, it was possible to "create" clusters by statistical manipulation. Careful attention must be paid to this type of situation in other descriptions in the literature of leukemia clusters.

Despite the desirability of a continued search for infectious patterns in the distribution of human leukemia, the observation most suggestive of a viral influence in human cancers is the geographic distribution reported for Burkitt's lymphoma in Africa.

Proposed Course of Project:

A further refinement of the Ederer-Myers-Mantel procedure for detecting clustering will be applied to the Los Angeles data when a suitable computer program is completed.

Publications

Glass, A. G., and Miller, R. W.: Significance of leukemia clusters. J. Pediatrics. In Press.

Fraumeni, J. F., Jr., and Miller, R. W.: Epidemiology of human leukemia: Recent observations. J. Nat. Cancer Institute 38: 593-605, 1967.

Miller, R. W., and Fraumeni, J. F., Jr.: "Leukemia houses". Ann. Intern. Med. 67: 674-676, 1967.

Serial No. NCI-4373
1. Epidemiology Branch, OASDD, Etiology
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Role of the Immune System in the Pathogenesis of Human Malignancies and Congenital Defects

Previous Serial Number: None

Principal Investigator: Arthur Oleinick, M.D.

Other Investigators: None

Cooperating Units: U.S.P.H.S. Hospital, Carville, Louisiana; Rheumatology Units, Departments of Internal Medicine: Columbia University, University of Michigan, and the New York University

Man Years:

Total:	1.4
Professional:	1.2
Other	0.2

Project Description

Objectives:

1. To determine the cancer mortality risk and, more specifically, the risk of leukemia/lymphoma among patients with leprosy, a disease associated both with a defect in cellular immunity and chronic stimulation of the immune system.

2. To determine the extent of the aggregation of lymphoproliferative malignancies and congenital defects among siblings and offspring of patients with systemic lupus erythematosus (SLE), a disease characterized by marked abnormalities of the immune system.

Methods Employed:

1. Mortality status and cause of death were ascertained for the 953 patients with leprosy whose first admission to the U.S.P.H.S. Hospital at Carville, Louisiana, occurred between 1939 and 1963. Follow-up was complete for over 90% of the study group. The observed number of cancer deaths was compared with the number expected based upon U.S. cancer mortality rates.

2. Of 260 patients with systemic lupus erythematosus contacted, 76% cooperated by returning a comprehensive family history questionnaire. Causes of death for siblings and offspring were ascertained from death certificates while congenital defects were ascertained from the questionnaire, supplemented by medical inquiry. The number of lymphoproliferative malignancies and congenital defects observed will be compared with that expected from U.S. mortality rates and previous reports.

Major Findings:

1. The cancer mortality risk of leprosy patients closely approximates that of the U.S. population. There is no excess of leukemia or lymphoma deaths in the study population.

2. This portion of the project is currently under analyses.

3. Accumulation of the above data resources permitted several additional studies that yielded the following findings:

   A. Survival among leprosy patients in the sulfone era approximates that of the U.S. population, except for special subgroups.

   B. Sex ratio analysis involving the siblings and offspring of the patients with SLE implicated an environmental factor in the pathogenesis of the disease. The conclusion was supported by a subsequent analyses of the mortality experience of the family members.

Significance to Bio-Medical Research:

The present studies attempt to establish the contribution of the immune system to the pathogenesis of human malignancies. The analysis of the cancer mortality risk among patients, or their family members, with diseases associated with an abnormal immune status may shed light on the normal immune system's function in this area. In addition, the establishment of the data resources required to consider the cancer risk has permitted studies which are highly relevant to the fields of leprosy therapy and rheumatic disease etiology.

Proposed Course of Project:

The analysis of the familial aggregation of lymphoproliferative diseases and congenital defects among patients with SLE should be completed during the coming year.

Publications

Oleinick, A.: Survival among leprosy patients with special consideration of cancer as a cause of death. Int. J. Leprosy. In press.

Serial No. NCI-4367
1. Epidemiology Branch
2. Epizootiology Section
3. East Lansing, Michigan

PHS - NIH
Individual Project Report
July 1, 1967 - June 30, 1968

Project Title: Bovine Population Studies

Previous Serial Number: NCI 4367

Principal Investigator: W. A. Priester, D.V.M.

Other Investigators: G. W. Robinson, G. H. Conner (Michigan State University)

Cooperating Units: Michigan State Dairy Herd Improvement Association (DHIA), East Lansing, Michigan, Windrow Farms, Metamora, Michigan

Man Years:

Total:	.2
Professional:	.1
Other:	.1

Project Description:

Objectives:

1. To characterize a dairy cow population by herd size and age.
2. To establish levels of mortality and disposal in a dairy cow population.

Methods Employed: A sample (approximately 10,000) of the central Michigan dairy cow population will be followed, by month, through a one-year period (January 1 - December 31, 1964). Attrition by month, cause, and individual will be tabulated. Individual animal status reports, submitted monthly to DHIA, will be used as the data source.

An optimally-managed purebred herd (Windrow Farms, Metamora, Michigan) will be followed through a five-year period (1961-1966). Mortality and disposal data will be recorded to establish the probable lower limit of mortality experience for the study area.

Major Findings: These will not be available until the above has been completed.

Significance to Bio-Medical Research and the Program of the Institute: Basic animal population data, essential in many epidemiologic analyses, are generally unavailable. It has been necessary for Epizootiology Section to assemble reference populations to bridge the data gap. This project is simply a continuation of animal reference population construction.

Proposed Course of Project: Using identical procedures, and samples from the same central Michigan counties, but for a later year, it will be possible to examine dairy cow population changes, if any, across time. We are currently attempting to account for a high loss to follow-up (30%/year) noted in the 1964 data.

Serial No. NCI-4368
1. Epidemiology Branch
2. Epizootiology Section
3. East Lansing, Michigan

PHS - NIH
Individual Project Report
July 1, 1967 - June 30, 1968

Project Title: Preliminary Study of Veterinary Medical Data Program (VMDP) Tumor Case Accumulation

Previous Serial Number: NCI 4368

Principal Investigator: W. A. Priester, D.V.M.

Other Investigators: None

Man Years:

Total:	.4
Professional:	.2
Other:	.2

Project Description:

Objectives:

1. To establish level of validity of tumor cases submitted to VMDP.
2. To determine the cause of inconsistencies found.
3. If necessary, to recommend control procedures that will insure a higher level of validity in the VMDP.

Methods Employed: Tumor data stored in VMDP were compared with original case folders at selected participating institutions. Points of especial interest were:

1. Basis for diagnosis.
2. Accuracy of coding diagnosis.
3. Transcribing error.

Major Findings:

1. Tumor diagnoses were reliable except that the level of confirmation by histopathology was under-reported.
2. The nature of the coding system used in reporting tumors necessitates continuing control of VMDP tumor data by examination of cumulative records by case number.
3. Transcribing error is negligible.

Significance to Bio-Medical Research and Program of Institute: As stored VMDP information reaches sufficient volume to permit quantitative analysis of special categories, it is essential that the data be tested for reliability. When the general level of reliability has been established, it will be possible, in utilizing stored data directly, to adjust for the level of probable error.

Proposed Course of Project: From the data accumulated during the project, began in December 1966, it appears necessary to continue verification in original records on an indefinite basis. Although general control procedures for VMDP have been in effect for some time, the importance of tumor data to Epizootiology Section justifies particular attention to the category.

Serial No. NCI-4369
1. Epidemiology Branch
2. Epizootiology Section
3. East Lansing, Michigan

PHS - NIH
Individual Project Report
July 1, 1967 - June 30, 1968

Project Title: Canine Lymphoma (CL): Relative Risk in the Boxer Breed

Previous Serial Number: NCI 4369

Principal Investigator: W. A. Priester, D.V.M.

Other Investigators: None

Cooperating Units: Schools of Veterinary Medicine at Auburn University, University of California, University of Georgia, and Michigan State University

Man Years:

Total:	.1
Professional:	.1
Other:	.0

Project Description:

Objectives:

1. To test the hypothesis that the Boxer breed is at an excess risk of CL.
2. To determine general magnitude of Boxer risk of CL.

Methods Employed: 240 cases of CL from three regions, Midwest, South, and West Coast were compared with control populations from the same regions.

Major Findings: The Boxer breed had a significantly ($P < .0005$) higher risk of CL than all other purebreds combined. The summary Boxer risk of CL was approximately six.

Significance to Bio-Medical Research and Program of the Institute: Identification of breeds at higher risk to CL may provide clues as to possible etiology. Similarity of certain physical characteristics of high risk breeds might indicate an environmental effect;

closely related high risk individuals might indicate a genetic or strain effect. In addition, identification of high risk breeds may be of value in future laboratory or field study design.

Proposed Course of Research: As the Boxer breed proved to be at high risk of CL, detailed studies of possible environmental or genetic factors involved may be undertaken, as suggested above.

Publications

Priester, W. A., "Canine Lymphoma: Relative risk in the boxer breed", J. Nat. Cancer Institute 39, # 5, November 1967.

Serial No. NCI-4370
1. Epidemiology Branch
2. Epizootiology Section
3. East Lansing, Michigan

PHS - NIH
Individual Project Report
July 1, 1967 - June 30, 1968

Project Title: Canine Population Study

Previous Serial Number: NCI 4370

Principal Investigator: G. W. Robinson, D.V.M.

Other Investigators: R. A. Tjalma, D.V.M.

Cooperating Units: Data Processing Center, Michigan State University

Man Years:

Total:	1.1
Professional:	.8
Other:	.3

Project Description:

Objectives: To collect, store, and analyze data describing normal dog populations, with the primary goal being the establishment of reference animal populations for use in comparative cancer research activities.

Methods Employed: Records of registered dog populations obtained from official municipal and county agencies in Alabama, Massachusetts, Michigan, and Ohio were analyzed. These records were of three types: individual licenses, census listings, and vaccination certificates. The type and year of the record obtained were determined by what was available at the time of the request and the cooperation of local agencies. Age, breed, and sex were abstracted and compiled, transferred to IBM punch cards, processed, and stored for subsequent retrieval.

Recently, three counties in Michigan and one county in Alabama were sampled a second time. Samples were also collected from seven counties in New York. Pertinent data were abstracted and added to the existing Canine Population Deck at the Michigan State University Data Processing Center.

Major Findings:

1. Data used on studies of canine bone sarcoma and lymphosarcoma.
2. Differences observed in the age, breed and sex composition of regional population samples.

Significance to Bio-Medical Research: Productive, analytic epizootiologic studies are seriously limited by the lack of data on representative animal populations. There is a definite need for the establishment and maintenance of reference animal populations as an aid in comparative research in cancer and medicine. Although license and census data are deficient in absolute numbers of dogs, characterization of these populations facilitates calculation of relative risk of disease by age, breed and sex.

Proposed Course of Project: Project terminates June 30, 1968.

Publications

Robinson, G. W.: Characterization of several canine populations by age, breed, and sex. J. Amer. Vet. Med. Assn. 151: 1072-1078, October 15, 1967.

Serial No. NCI-4371
1. Epidemiology Branch
2. Epizootiology Section
3. East Lansing, Michigan

PHS - NIH
Individual Project Report
July 1, 1967 - June 30, 1968

Project Title: National Cancer Institute Veterinary Medical Data Program

Previous Serial Number: NCI 4371

Principal Investigator: W. A. Priester, D.V.M.

Other Investigators: R. A. Tjalma

Cooperating Units: Schools and Colleges of Veterinary Medicine: Michigan State University, University of Missouri, University of Minnesota, Iowa State University, Florida Diagnostic Laboratories, Ontario Veterinary College, Purdue University, University of Georgia, Kansas State University, University of Illinois, University of California, Royal Veterinary College, London, and University of Agriculture, Czechoslovakia.

Man Years:

Total:	1.5
Professional:	.7
Other:	.8

Project Description:

Objectives: To provide animal disease data as a research resource. To coordinate the program with related programs primarily in area of disease nomenclature, terminology and code. To develop ways and means of data utilization applicable to comparative medical studies.

Methods Employed: Standard data processing methods. Details are described in related Project Reports and Contract Narratives of Epizootiology Section.

Major Findings: Not applicable as this is primarily a service activity. The National Cancer Institute Veterinary Medical Data Program is currently processing data from 9 veterinary colleges in this country, 1 in Canada, and 2 in Europe. A modified program has been developed for diagnostic laboratories (pilot study Florida

Diagnostic Laboratories). The program has been endorsed and implemented in Europe under auspices of WHO. Additional institutions will be added in accordance with available funds in an orderly and logical sequence. For the first time, veterinary medicine and the medical sciences will have benefit of a national/international animal disease data system.

Significance to Bio-Medical Research: The comparative (veterinary) medical data retrieval system instituted at College of Veterinary Medicine, Michigan State University on a pilot basis (PH 43-64-85) was undertaken to design a system for collection, storage and retrieval of veterinary medical clinical data. The program is now fully operative and has been extended to nine additional veterinary colleges, (University of Minnesota, University of Missouri, Iowa State University, Ontario Veterinary College, Purdue University, University of Georgia, University of California, University of Illinois, and Kansas State University). Establishment and maintenance of reliable sources of veterinary medical data will provide a research resource, hitherto unavailable, that is applicable not only to cancer research but to research areas such as infectious diseases, parasitic diseases, and gerontological diseases. Continued NCI Epizootiology Section research function will depend upon continued availability of valid domestic animal disease data, particularly as regards neoplastic disease.

Proposed Course of Project: It is anticipated that this project will continue for a minimum of two years. The Florida Diagnostic Laboratory assumes responsibility for processing their data locally April 1, 1968.

Date Initiated: October 22, 1965

Current annual level: $17,600

Publications

Peters, J. A., and Martin, F. B.: "The Veterinary Medical Data Program: A Systematic Method of Collection, Storage, Manipulation and Recall of Animal Disease Data". MSU Veterinarian 26, # 3, 1966.

Peters, J. A.: "Veterinary Clinical Data: An Epidemiologic Research Resource". Am. J. of Public Health 56, # 11, November 1966.

Peters, J. A.: "Standard Nomenclature and Data Retrieval in Veterinary Medicine". American J. of Veterinary Research 28, # 123, March 1967.

Other Presentations

Peters, J. A.: "Standard Nomenclature and Data Retrieval in Veterinary Medicine". AVMA Annual Meeting, Research Section, Louisville, July 1966.

Peters, J. A.: "Veterinary Clinical Data: An Epidemiologic Research Resource". APHA Annual Meeting, Epidemiology Section, Chicago, Illinois, October 1965.

Serial No. NCI-4374
1. Epidemiology Branch
2. Epizootiology Section
3. East Lansing, Michigan

PHS - NIH
Individual Project Report
July 1, 1967 - June 30, 1968

Project Title: Comparison of Licensed and Hospital Dog Populations

Previous Serial Number: None

Principal Investigator: G. W. Robinson, D.V.M.

Other Investigators: None

Cooperating Units: Data Processing Center, Michigan State University

Man Years:

Total:	0.3
Professional:	0.2
Other:	0.1

Project Description:

Objectives: To determine: (a) whether dogs enter veterinary hospitals in the same proportions as they are distributed by age, breed and sex in the licensed population; and (b) the extent of differences in relative risk of cancer when calculated on the basis of a county licensed population and a hospital population within the same county.

Methods Employed: Age, breed and sex were abstracted from individual licenses of dogs registered in Ingham County, Michigan, during the year 1964 and from case records of all individual dogs admitted to Michigan State University Veterinary Hospital from March 1964 through February 1965. Data were analyzed for proportionate distribution of dogs in both populations and relative risks of cancer was computed for several selective breeds utilizing each population as a base.

Major Findings:

1. A county licensed dog population and a hospital dog population within the same county were found to differ in age, breed and sex distribution.

2. Two of 3 selected breeds had significant differences when relative risks based on the two populations were compared.
3. Risk of cancer was significantly higher in Boxers, both males and females, than for all other breeds, using both population bases.

Publications

Robinson, G. W.: A comparison of licensed and hospital dog populations. J. Amer. Vet. Med. Assn. 152 (8): April 15, 1968.

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, ETIOLOGY
Fiscal Year 1968

Office of the Chief

HIROSHIMA UNIVERSITY SCHOOL OF MEDICINE (PH43-66-61)

Title: Oncogenic and Teratogenic Effects of Mustard Gas in Man

Contractor's Project Director: Dr. Sunao Wada

Project Officer (NCI): Dr. Robert W. Miller

Objectives: To study the effects of chronic occupational exposure to mustard gas on oncogenesis and reproduction.

Methods Employed: About 2,000 persons were employed between 1929 and 1945 to a mustard-gas factory on Okuno-jima, an island about 50 miles from Hiroshima. These workers usually wore incompletely protective or no protective clothing, and many of them suffered hemoptysis during employment. Study of several hundred workers to date has revealed that among 172 deaths, 28 were due to cancer of the respiratory tract. The data suggest also an excess of cancer of the liver, esophagus and skin. Under this contract the study would be extended to include as many former workers who still live in the Takehara area as can be traced by intensive medical detective work to determine causes of death and morbidity in a search for neoplasia of the respiratory tract and of other organ systems, and, since nitrogen mustard is a powerful teratogen and mutagen in the animal laboratory, a search will also be made for an adverse effect of mustard-gas exposure on the progency of the workers.

Since mustard gas is a strong teratogenic agent in the animal laboratory, it will be of interest to appraise the outcome of pregnancies which occurred while women worked at desk jobs in the factory to determine if the frequencies of miscarriage or major malformations were substantially higher than normal expectation.

Major Findings: A marked excess of oral and respiratory-tract cancers has been demonstrated among former mustard gas workers. No effect on reproductive performance has been detected.

Significance to NCI Program and Bio-Medical Research: The results obtained will contribute to laboratory and epidemiologic studies into the etiology of cancer and congenital malformations. These results will be important not with respect to mustard-gas exposure, which is uncommon, but for the broader biologic implications. (1) An excess of neoplasia in several tissues will add to the evidence that chemical carcinogens in man are not organ-specific. (2) There will be new information as to the length of latent period which can be compared with those of other human carcinogens. (3) Characteristics of persons who developed neoplasia as compared with those who did not may reveal circumstances other than mustard-gas exposure which contribute to neoplasia. (4) In a single study the oncogenic and teratogenic effects of a chemical will be evaluated, a circumstance which brings attention again to the overlap between these two abnormal processes. (5) The genetic concept of the influence of paternal exposure to a mutagenic agent on the sex ratio of offspring, as described above, can be put to a good test because the exposures in this instance were great and prolonged. (6) Because mustard gas imitates the effects of ionizing radiation, whatever is learned from the study of the former factory workers may be applicable in the study of the delayed consequences of radiation which coincidentally is being conducted nearby among the survivors of the atomic bomb.

Proposed Course of Project: Studies will be continued and interim report of results to date should be ready by June 1968.

Date Contract Initiated: October 19, 1965

Current Annual Level: $10,747

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, ETIOLOGY
Fiscal Year 1968

Ecology Studies Section

WESTERN RESERVE UNIVERSITY (PH43-64-524)

Title: Mortality Experience of Children Who Were Inadvertently Inoculated with SV-40 Very Early in Life

Contractor's Project Director: Dr. Martha L. Lepow

Project Officer (NCI): Charles R. Stark, M.D.

Objectives: To determine whether the inoculation of human infants with SV-40 at birth is associated with an increased mortality due to malignant neoplasms.

Methods Employed: The mortality experience of 1,026 Negro children, born in Cleveland, Ohio, between December 1959 and May 1962 and inoculated with SV-40 shortly after birth, will be followed through time. The location and name of each child will be determined annually. Death due to cancer among cohort-children will be discovered through the use of a continuously up-dated census of Cleveland school children and from the vital records of the State of Ohio and other states to which the children have migrated. The frequency of cancer deaths among cohort-children will be compared with that for all Negro children in the State of Ohio. The Poisson distribution will be used as a basis for deciding whether the cohort-children suffer an excess cancer mortality.

Major Findings: As of December 31, 1965, eleven deaths had occurred in study children. Eight of the deaths were among the 572 newborn study children who received relatively large amounts of SV-40 in orally administered Sabin type 1 poliovaccine. Three of the deaths were among the 514 newborn study children who received very little or no SV-40 in Purivax killed poliovaccine given I.M. or Sabin type 2 poliovaccine given orally. None of the deaths could be attributed to the inadvertent inoculation with SV-40

Significance to NCI Program and Bio-medical Research: Previous investigations (by the Epidemiology Branch) of school-age children who were inadvertently inoculated with SV-40 will be extended. The known importance of neonatal inoculation in the causation of many viral neoplasms of animals suggests the possibility that the cancer mortality experience of children inoculated at birth might be different from that of children inoculated at school age. The present study will complement our present knowledge in this area.

Proposed Course of Project: The Cleveland school census and the vital records of Ohio and other States will be used to monitor deaths among study children for an indefinite period of time. An interim report of results to date should be ready by June 1968.

Date Contract Initiated: March 9, 1964

Current Annual Level: $9,251

UNIVERSITY OF PENNSYLVANIA (PH43-62-432)

Title: Development and Application of Scanning Photometry and Digital Computers in Cytomorphology and Diagnosis

Contractor's Project Director: Mortimer L. Mendelsohn, M.D., Ph.D.

Project Officer (NCI): Charles R. Stark, M.D.

Objectives: This effort seeks to identify computer-derived measurement parameter systems capable of taxonomic description of morphological patterns. Specific problems which could be addressed are numerous. However, the scope of the first five years of this contract will be limited to study of (1) normal leukocytes and leukemia, and (2) normal and abnormal chromosomes.

Methods Employed: A photometer constructed at Airborne Instruments Laboratory under a National Cancer Institute contract scans individual cells, transforms the resulting photometric signal into digital form and records the digitalized data on magnetic tape in a form suitable for input to a digital computer. An extensive library of programs selectively activated by parameter cards submitted by the investigators causes the computer to convert input data into a measure of a given parameter or combination of parameters which in turn are analyzed and evaluated as descriptors of the morphological pattern under study. This process will be repeated, altered and refined until a parameter or group of parameters constituting a computer-derived taxonomic system for the morphologic patterns under study is identified.

Major Findings: The contract has found that optical density frequency distributions for normal human leukocytes are unique for the various classically recognized leukocytes. The contractor has also found that a multi-parameter measurement system using optical density input data from the CYDAC adequately classifies human chromosomes into 11 groups.

Significance to NCI Program and Bio-medical Research: Rapid, unerring, quantitative measurements of numerous kinds of biological patterns is essential to the expanding investigations of cancer etiology under way in the National Cancer Institute. For the epidemiologists the measurement and classification of leukemic cells would open up vast new areas for epidemiologic investigations. Similarly, more precise measurement and classification of human chromosomes would provide an additional parameter in epidemiologic studies as well as carcinogenesis studies. For the viral oncologist, automated measurement of DNA content and virus morphology would provide him with more time for reflection. For the molecular biologist, quantitative measurements of DNA content or other histochemical parameters would be of similar value. As well as offering the promise of performing measurements of various kinds, the CYDAC system shows promise, as in the case of human chromosomes, of refining existing human-based taxonomies. Thus, the unique ability of the computer to unerringly organize vast quantities of measurements, if properly guided and assisted by the human investigator, could provide us with perceptual capabilities currently beyond our reach.

Proposed Course of Project: The published reports of progress establish the scientific merit of the work being done under this contract. However, the basic research currently performed does not seem to have direct relevance to the Epidemiology Branch program at present or in the next few years. Since some other mechanism of support seems appropriate, the contract will be terminated in Fiscal Year 1969.

Date Contract Initiated: March 31, 1962.

Current Annual Level: $142,860

HARVARD SCHOOL OF PUBLIC HEALTH (PH43-67-732)

Title: Leukemia Following Radiotherapy for Benign Gynecological Disease

Contractor's Project Director: Brian MacMahon, M.D.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D.

Objectives: To determine whether women who received radium therapy for benign gynecologic diseases have experienced an increased incidence of leukemia or cancers in heavily irradiated sites, as contrasted with the experience of the general population. To evaluate the dose-response relationship for leukemia and other cancers. To assess the effects of radiation castration on the risk of breast cancer and of atherosclerosis.

Methods Employed: Contractor will supervise the abstracting of data from the medical records located in radiology departments and records rooms, at hospitals in Connecticut and Massachusetts. Records on approximately 8,000-10,000 women receiving radiotherapy will be abstracted for information on radiation-exposure factors, medical conditions, demographic characteristics, and follow-up information. These data will be coded and placed on punch cards. By name search of files at the State vital-statistics and cancer-registry departments and by follow-up letters, information will be obtained on the outcome of patients in the study group. Follow-up information will be coded and placed on punch cards, with computer analysis performed at NIH.

Major Findings: Not available as yet.

Significance to NCI Program and Bio-medical Research: The mechanism of induction of human leukemia by ionizing radiation is still poorly understood. Since the effects of low doses can rarely be assessed directly, knowledge of this mechanism is important for such practical matters as the estimation of low-dose effects from observations made on populations exposed to high doses. A recent finding of considerable significance to these considerations is that women exposed to high doses of radiation in the treatment of pelvic malignancy do not have increased frequency of leukemia, even though the mean marrow doses received were comparable to those received by other study groups that have shown substantial leukemia increases. On the other hand, there are suggestions, based on relatively small numbers, that women receiving the lower doses associated with the treatment of benign gynecological conditions do have increased leukemia incidence. It seems important, therefore, to study adequate numbers of women having radiological menopause and other medium-dose gynecologic exposures to determine whether they experience an increased frequency of leukemia, and, if so, whether a dose-response relationship exists within the range of doses used in such procedures.

Proposed Course of Project: It is anticipated that this project will be completed by June 1969.

Date Initiated: March 8, 1967

Current Annual Level: $16,680

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, ETIOLOGY
Fiscal Year 1968

Special Cancer Studies Section

WAYNE STATE UNIVERSITY (PH43-66-53)

Title: Epidemiologic Studies of Occupational Exposure to Cutting Oil Mists

Contractor's Project Director: Thieu L. Nghiem, M.D., Dr.P.H.

Project Officer (NCI): Frank E. Lundin, Jr., M.D.

Objectives: To determine whether occupational exposure to cutting oil mists produces significant disease of the respiratory tract. In particular, evidence of impaired pulmonary function, chronic bronchitis, obstructive emphysema, chronic pneumonitis, pulmonary fibrosis, other distinctive pathologic changes, and respiratory cancer is sought.

Methods Employed:

1. A mortality study is made of approximately 8,000 subjects whose employment (for one or more years between 1937 and 1966) exposed them to cutting oil mists. Length, time, and intensity of exposure to cutting oil mists is determined from employment records. Death certificates are located through retirement and insurance records and the Social Security Administration. Using a life table technique, person years at risk by age, race and year are determined. The cause-specific mortality among the study group, classified according to the duration of exposure to cutting oil mists, is compared with that for the general population of the area and with unexposed employees of the same industry.

2. Approximately 1,000 employees currently exposed to cutting oil mists and an equal number of control subjects complete questionnaires which include smoking and occupational histories, and have chest x-rays, pulmonary function tests, and complete histories and physicals. (The participating industry provides the medical personnel for this aspect of the study).

3. A final subgroup of approximately 2,000 exposed workers who left employment after January 1, 1956 are compared with a group of unexposed employees with regard to abnormalities of their chest x-rays and selected diseases requiring hospitalization.

Major Findings: None

Significance to NCI Program and Bio-medical Research: Carcinogenic hazards for man can be identified by epidemiologic studies of the effects of selected occupational exposures. In this way preventive measures will be undertaken. This study heralds a program of epidemiologic studies which will be initiated provided the following conditions obtain: (1) experimental evidence of carcinogenic activity, (2) large numbers of employees exposed over a period of years, and (3) collaboration between NIH, industry, and possibly, academic institutions.

Proposed Course of Project: As originally outlined the study required 3 to 5 years for completion. In the absence of a full-time epidemiologis initiation of the study was delayed; however, an epidemiologist was recruited in August 1966 but was killed in an automobile accident in May 1967. In August 1967 another epidemiologist (Dr. Nghiem) was recruited and a nurse epidemiologist (Winifred Mendez) was assigned to the project by the Division of Environmental Health Sciences (DEHS). In November the project director (Dr. Arthur J. Vorwald) suffered a stroke and the epidemiologist was made project director. With these difficulties it is possible that completion of the project will be somewhat delayed. This contract will be transferred to DEHS in December 1968.

Date Contract Initiated: August 10, 1965

Current Annual Level: $105,000

CONTRACT NARRATIVE
(Summary of Personal Service Contracts)

Epizootiology Section

Personal Service Contracts between the Epizootiology Section and Colleges of Veterinary Medicine at University of Missouri, Michigan State University, Iowa State University, Ontario Veterinary College, and the University of Georgia, University of Illinois, and Kansas State University.

Title: Purchase Veterinary Case Data.

Objectives: To add to volume of stored data, available to Epizootiology Section, in the Veterinary Medical Data Program. To further encourage adoption of standard nomenclature and coding of animal disease data. To make available to veterinary medicine, comparative medical research and the scientific community the developments of the Veterinary Medical Data Program.

Methods Employed: Purchase of completed veterinary medical case abstracts in terms of National Cancer Institute Veterinary Medical Data Program (as detailed in related Contract Narratives).

Major Findings: None, as this is primarily a service activity.

Significance to NCI Program and Bio-Medical Research: Increase store of data in the Veterinary Medical Data Program. Of particular importance in terms of study of low prevalence diseases (cancer, etc.), geographical distribution and absolute numbers.

Proposed Course of Project: Minimum of two years.

Dates Initiated:

Dates Initiated:	Michigan State University	November, 1965
	University of Missouri	February 1967
	Iowa State University	October 1965
	Ontario Veterinary College	July 1966
	University of Georgia	January 1967
	University of Illinois	January 1968
	Kansas State University	January 1968

Current Annual Level: $17,500.00 ($2,500.00 maximum each school).

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, ETIOLOGY
Fiscal Year 1968

Epizootiology Section

PURDUE UNIVERSITY (PH 43-66-957)

Title: Implementation and Demonstration of Veterinary Medical Records, Data Retrieval and Standard Nomenclature Systems.

Contractor's Project Director: Dr. H. E. Amstutz, Head, Department of Veterinary Clinics, Purdue University

Project Officer (NCI): William A. Priester, D.V.M.

Objectives: The NCI comparative medical data program, as designed and tested, has proven efficient and productive, and has developed implication beyond that originally visualized. Extension of the program to include additional institutional veterinary medical clinical facilities is necessary to further data program development and evaluation, and is prerequisite to future Epizootiology Section function.

Methods Employed: The Veterinary Medical Data Program originally developed at the College of Veterinary Medicine, Michigan State University has been used, where indicated, as a model for extension of the program to additional institutions. The stepwise evolution of the program at Purdue, based on the Michigan State experience, was as follows:

1. Redesign and adaptation of present interdepartmental and clinical record forms and procedures to support automated data storage and retrieval operation. The responsibility for this step was assumed by Purdue University, School of Veterinary Medicine Records Committee and the medical records librarian recruited for the project.
2. Following development of a suitable internal system, a standard precoded case abstract form is completed for each veterinary clinic patient record. The principal investigator, through the records librarian, will be responsible for the accuracy and completeness of case abstracts.
3. Abstracts will be processed and complete reports returned to School of Veterinary Medicine, Purdue University by Epizootiology Section, NCI.

Major Findings: The Veterinary Medical Data Program, initiated by Epizootiology Section, NCI, has been in full scale operation since February 1, 1964. Evaluation has demonstrated the program to be of adequate capacity, refinement, and sensitivity to support additional data sources. Inclusion of additional schools has served to capitalize on previous experience at Michigan State University, increasing input into the total data program with minimum time lag.

The entire data activity is considered essential to effect future function of Epizootiology Section, NCI. Further, the establishment and maintenance of reliable sources for veterinary medical data will open many new opportunities, not only in cancer research and all its ramifications, but also in other research areas such as infectious disease, parasitic infestation, degenerative disease, and aging.

Significance to NCI Program and Bio-Medical Research: This proposal has constituted a feasible and efficient approach to the problem of generating and securing comparable baseline data on domestic animal diseases. Never have comparative medical investigators had the advantage of access to meaningful veterinary clinical data. In addition to providing important information on the observed occurrence, diagnosis, and behavior of selected animal diseases such as cancer and cardiovascular disease, the data program provides opportunity, within the veterinary area, to design and organize analytic, progressive research on a much sounder basis than has been possible. Extension of the Veterinary Medical Data Program has served to make available a greater quality of comparable data, permits evaluation of the data system as a multi-source program, and promotes firm organization and general adoption of the data system.

Proposed Course of Project: Research contract will terminate December 1968, and convert to personal service contract ($2,500.00 per year maximum).

Date Initiated: June 28, 1966

Current Annual Level: $7,300.00

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, ETIOLOGY
Fiscal Year 1968

Epizootiology Section

MICHIGAN STATE UNIVERSITY (PH 43-66-75)

Title: Data Processing Service for the Veterinary Medical Data Program

Contractor's Project Director: Francis B. Martin

Project Officer (NCI): William A. Priester, D.V.M.

Objectives: To routinely produce (monthly) machine listings and summaries utilizing medical case abstracts as source document. To provide special listings, summaries, etc., as directed by project officer. To provide data processing support and technical consultation for revision of Standard Nomenclature of Veterinary Diseases and Operations. To make available the services of data processing for such special activities as may be developed by Epizootiology Section.

Methods Employed: Contractor will furnish all necessary labor, materials, and facilities to obtain above objectives in keeping with accepted methods and procedures of data processing. Available equipment with support personnel includes IBM Keypunch, Verifiers, Sorters, Calculators, Collators, Interpreters, Reproducers, Tabulators, one 360, 70K computer system.

Major Findings: Not applicable, as this is primarily a service contract.

Significance to NCI Program and Bio-Medical Research: The comparative (veterinary) medical data retrieval system instituted at College of Veterinary Medicine, Michigan State University on a pilot basis (PH 43-64-85) was undertaken to design a system for collection, storage and retrieval of veterinary medical clinical data. The program is now fully operative and has been extended to nine additional veterinary colleges (University of Minnesota, University of Missouri, Iowa State University, Ontario Veterinary College, Purdue University, University of Georgia, University of Illinois, University of California, and Kansas State University). Establishment and maintenance of reliable sources of veterinary medical data will provide a research resource, hitherto unavailable, that is applicable not only to cancer research but to research areas such as infectious diseases, parasitic diseases, and gerontological diseases. Continued NCI Epizootiology Section research function will depend upon continued availability of valid domestic animal disease data, particularly as regards neoplastic disease.

Proposed Course of Project: It is anticipated that this project will continue to January 1969, during which time a phase-in to NIH processing will be effected.

Date Initiated: October 22, 1965

Current Annual Level: $17,930.00

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, ETIOLOGY
Fiscal Year 1968

Epizootiology Section

UNIVERSITY OF MINNESOTA (PH 43-65-60)

Title: Testing and Evaluation of the Veterinary Medical Data Program

Contractor's Project Director: W.T.S. Thorp, D.V.M., Dean, College of Veterinary Medicine, University of Minnesota

Project Officer (NCI): William A. Priester, D.V.M.

Objectives: To further test and develop the NCI Comparative Medical Data Program particularly those aspects concerned with institutional differential when used as a component of a multi-source input. To evaluate the comparability of multi-source data. To expand the volume of stored data for analysis and to obtain geographical distribution of input information.

Methods Employed: The Veterinary Medical Data Program now in operation at the College of Veterinary Medicine, Michigan State University has been used, where indicated, as a model for extension of the program to the School of Veterinary Medicine, University of Minnesota. The stepwise evolution of the program at Minnesota, based on the Michigan State experience, was as follows:

1. Redesign and adaptation of present interdepartmental and clinical record forms and procedures to support automated data storage and retrieval operation. The responsibility for this step was assumed by University graduate medical records librarian recruited for the project.
2. Following development of a suitable internal system, a standard precoded case abstract form is completed for each veterinary clinic patient record. The principal investigator, through the records librarian, will be responsible for the accuracy and completeness of case abstracts.
3. Abstracts will be processed and complete reports returned to School of Veterinary Medicine, University of Minnesota by Epizootiology Section, NCI.

Major Findings: The Veterinary Medical Data Program, initiated by Epizootiology Section, NCI, has been in full scale operation since February 1, 1964. Evaluation has demonstrated the program to be of adequate capacity, refinement, and sensitivity to support additional data sources. Inclusion of additional schools has served to capitalize on previous experience at Michigan State University, increasing input into the total data program with minimum time lag.

The entire data activity is considered essential to effective future function of Epizootiology Section, NCI. Further, the establishment and maintenance of reliable sources for veterinary medical data will open many new opportunities not only in cancer research and all its ramifications, but also in other research areas such as infectious disease, parasitic infestation, degenerative disease, and aging.

Significance to NCI Program and Bio-Medical Research: This proposal has constituted a feasible and efficient approach to the problem of generating and securing comparable baseline data on domestic animal diseases. Never have comparative medical investigators had the advantage of access to meaningful veterinary clinical data. In addition to providing important information on the observed and cardio-vascular disease, the data program provides opportunity, within the veterinary area, to design and organize analytic, progressive research on a much sounder basis than has been possible. Extension of the Veterinary Medical Data Program has served to make available a greater quantity of comparable data, permits evaluation of the data system as a multi-source program, and promoted firm organization and general adoption of the data system.

Proposed Course of Project: Terminated August 1967, and converted to personal service contract ($2,500.00 per year maximum).

Date Initiated: November 1, 1964

Current Annual Level: None

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, ETIOLOGY
Fiscal Year 1968

Epizootiology Section

UNIVERSITY OF CALIFORNIA (PH 43-68-58)

Title: Implementation and Demonstration of Veterinary Medical Record, Data Retrieval and Standard Nomenclature Systems.

Contractor's Project Director: Dr. Joyce Goggin, D.V.M., M.P.H., School of Veterinary Medicine, University of California

Project Officer (NCI): William A. Priester, D.V.M.

Objectives: The NCI comparative medical data program, as designed and tested, has proven efficient and productive, and has developed implication beyond that originally visualized. Extension of the program to include additional institutional veterinary medical clinical facilities is necessary to further data program development and evaluation, and is prerequisite to future Epizootiology Section function.

Methods Employed: The Veterinary Medical Data Program now in operation at the College of Veterinary Medicine, Michigan State Universtiy has been used where indicated, as a model for extension of the program to the School of Veterinary Medicine, University of California. The stepwise evolution of the program at California, based on the Michigan State experience,was as follows:

1. Redesign and adaptation of present interdepartmental and clinical record forms and procedures to support automated data storage and retrieval operation. The responsibility for this step was assumed by University of California, School of Veterinary Medicine Records Committee and the medical records librarian recruited for the project.

2. Following development of a suitable internal system, a standard precoded case abstract form is completed for each veterinary clinic patient record. The principal investigator, through the records librarian, will be responsible for the accuracy and completeness of case abstracts.
3. Abstracts will be processed and complete reports returned to School of Veterinary Medicine, University of California by Epizootiology Section, NCI.

Major Findings: The Veterinary Medical Data Program initiated by Epizootiology Section, NCI, has been in full scale operation since February 1, 1964. Evaluation has demonstrated the program to be of adequate capacity, refinement, and sensitivity to support additional data sources. Inclusion of additional schools has served to capitalize on previous experience at Michigan State University, increasing input into the total data program with minimum time lag.

The entire data activity is considered essential to effective future function of Epizootiology Section, NCI. Further, the establishment and maintenance of reliable sources for veterinary medical data will open many new opportunities, not only in cancer research and all its ramifications, but also in other research areas such as infectious disease, parasitic infestation, degenerative disease, and aging.

Significance to NCI Program and Bio-Medical Research: This proposal has constituted a feasible and efficient approach to the problem of generating and securing comparable baseline data on domestic animal diseases. Never have comparative medical investigators had the advantage of access to meaningful veterinary clinical data. In addition to providing important information on the observed occurrence, diagnosis, and behavior of selected animal diseases such as cancer and cardiovascular disease, the data program provides opportunity, within the veterinary area, to design and organize analytic, progressive research on a much sounder basis than has been possible. Extension of the Veterinary Medical Data Program has served to make available a greater quantity of comparable data, permits evaluation of the data system as a multi-source program, and promotes firm organization and general adoption of the data system.

Proposed Course of Project: Terminates September 1968, anticipate renewal for one additional year.

Date Initiated: September 25, 1967

Current Annual Level: $9,086.00

Summary Statement
OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR
FOR CARCINOGENESIS, ETIOLOGY
July 1, 1967 - June 30, 1968

The Carcinogenesis Area, Etiology, is supported by two branches, the Chemistry and the Biology Branch. The Chemistry Branch, under the direction of Dr. Harry V. Gelboin, consists of four sections as follows: The Protein Section under Dr. A. Peacock, the Nucleic Acids Section under Dr. Dingman, the Cell Growth Regulation Section under Dr. Bader, and the Molecular Carcinogenesis Section with Dr. Gelboin as acting head. The aim of the activities of the scientists in that branch is the evaluation of significance of events on the cellular and subcellular level, which occur when carcinogens, or related chemicals, interact with cell organelles and enzyme systems with regard to carcinogenesis.

The Biology Branch, under the direction of Dr. Herbert Rapp, is divided into four sections, the Bioassay Section under Dr. R. Bates, the Carcinogen Screening Section under Dr. J. H. Weisburger, the Cytogenetics and Cytology Section under Dr. J. DiPaolo, and the Immunology Section under Dr. T. Borsos. In addition, the Office of the Chief houses the Molecular Separation Unit under Dr. H. Bond and the Ultrastructural Unit under Dr. D. Courington. The branch is focusing on the events in carcinogenesis involving the whole animal.

Details of the programs of each branch are given separately in this report and the following pages will describe the overall responsibilities of the Office of the Associate Scientific Director for Carcinogenesis, to explore all potential carcinogenic hazards to man in his environment, except those due to viruses.

The main function of this office is sustained alertness to new carcinogenic hazards which may be introduced into man's environment. In the past, clues to carcinogenic hazards for man were provided by observations and deductions made by industrial physicians, but mainly by epidemiologists. Much help has been given by scientists of the Epidemiology and Biometry Branches to this office in order to bridge the gap between laboratory experimentation in carcinogenesis and its meaningful extrapolation regarding the hazards to man.

Due to the rapid pace of industrial development of new products involving many chemicals, often involving cross-linking properties, it is imperative to evaluate the risk to man without the benefits of time and epidemiologic input, but to base the conclusions on bioassay results. These extrapolations from animal to man are often inadequate, suffer from lack of comparability, because of many dissimilarities in the two systems, but, nonetheless, these data should serve to alert scientists, physicians, plant managers, and the general public to a potential hazard. Hopefully, absolute proof of the carcinogenic hazard to man will never be forthcoming because precautions will have been taken to prevent cancer in man due to the particular agent.

To help in the extrapolation of laboratory data to the situation affecting the health of man, studies are being carried out to determine absorption, storage, metabolism, and detoxification of these suspect chemicals in a number of species and, where possible, similar studies are made on man. Of considerable help may be the rapid development of *in vitro* transformation of cells, which can be studied on animal as well as on human cell lines. A contract is being negotiated to explore this approach fully.

It may be suitable to discuss certain specific problems at this point. The cigarette smoking habit appears to be the greatest lung cancer risk, which has not been reduced by publizing the hazard, or explaining the risk to the teenagers. The primary direction taken by this office, therefore, is the development of a less hazardous cigarette. There is no lack of ideas or suggestions how to reach such a safer smoke, judging by the letters received from inventors or public-minded citizens, but the difficulties lie in the evaluation of the modification of the smoke regarding the real health benefits for the smoker, when they are based on the best, available bioassay techniques.

Several research projects have been under way for some time to give the foundation for the evaluation of a less hazardous cigarette. They deal with physico-chemical changes in tracheobronchial mucus in cats inhaling cigarette smoke, or with the combined effects of pulmonary infarction and cigarette smoke inhalation in rats, with lung cancer as the end point. Two other programs study the nature of the initiators and promoters in tobacco and tobacco smoke, focusing on polycyclic hydrocarbons, nitrosamines, oxides of nitrogen, free radicals, and other chemicals detected in smoke. In addition, two contracts are in the process of activation, which will study the interaction of cigarette smoke and certain inorganic dusts inhaled by rodents towards cancer induction, with special emphasis on asbestos, and salts of beryllium, cadmium, lead and other heavy metals. Exposure to such dusts is not restricted to industrial workers, but may effect large population groups.

Exploration of the lung cancer hazards for man also needs an evaluation of the air pollution hazard. Both kinds of urban air pollution, the oxidant and the reducing kind, are under study in combination with other factors to determine their contribution to lung cancer by life long inhalation studies in rodents.

Among the industrial chemicals, particular emphasis should be made of metallo-organic complexes which have been under study for several years and have revealed the existence of new carcinogenic compounds of much use in industry. Greater efforts in detecting these new hazardous compounds will have to be made.

The potential carcinogenic hazard of pesticides has been explored and is mentioned separately, because much attention has been focused on that risk in the past years. Our studies will be terminated shortly with the encouraging suggestion that the carcinogenic hazard is less than anticipated. Where carcinogenic activity was found, these observations generally confirmed previous findings on pesticides, which, by now, have been eliminated or restricted in use. Although the data are not complete as yet, the long survival of the experimental animals speaks in favor of the harmlessness of those chemicals which have been tested. However, our efforts to keep abreast of new developments in the field of agricultural and industrial chemicals motivated activation of two new contracts to determine potential carcinogenic activity of such newly developed chemicals.

The term "food contamination" has taken on different meanings in recent years, including, besides antioxidants, preservatives, pesticide residues, hormones, viruses, and bacteria, also a number of mycotoxins which appear to be powerful carcinogenic hazards. Our research efforts focus on mycotoxins in specific food staples. It includes foods consumed by peoples all over the world and particularly those foods deliberately exposed to fungi for purposes of fermentation.

The foodstuffs themselves, however, particularly vegetable oils, are also under study. They are fed to rodents in the diet as fresh, slightly oxidized or heated, as in deep-frying, to determine the carcinogenic risk and to identify the responsible chemicals. Irradiated fats are also under study. A new contract which will determine the cocarcinogenic properties of cyclopropenoid fatty acids, as found in cottonseed oil and others, will be initiated shortly.

Interest in the cocarcinogenic and carcinogenic properties of pharmaceutical agents has led to several contracts for exploration of parameters not covered by FDA regulation or research. Nitrofuran compounds, some of them used as antibacterials, have produced mammary tumors in female rats and minor chemical changes in the structure have led to carcinogenic activity for other organs such as the bladder, the intestines, the liver, or kidneys. Two new contracts, now under development, will deal with the potential carcinogenicity of chemotherapeutic agents, which are being developed primarily for the treatment of cancer patients, but may find other therapeutic usage and the potential cocarcinogenicity of certain oral contraceptives.

Other contractual activities deal with the development of specific chemicals as enzyme inhibitors, preparation of pure human complement fractions and the preparation of pure aflatoxins for use by qualified investigators. Another important service to the scientific community in cancer research has been the preparation and dissemination of Carcinogenesis Abstracts, whose publication is being reactivated at this time.

During this fiscal year, concerted efforts were made to find a successor to the Associate Scientific Director for Carcinogenesis which led to the successful recruitment of Dr. Umberto Saffiotti, who will take on his new duties shortly, thus making a smooth transfer of activities and responsibilities possible. During this period the duties of the Associate Scientific Director for Carcinogenesis have been excessive, because he was also charged with responsibility for the laboratory research activity at the National Environmental Health Sciences Center in the Research Triangle area in North Carolina, by agreement between the directors of the two organizations. He will transfer to DEHS at the end of this fiscal year.

Honors and Awards:

Hans L. Falk, Ph.D., Associate Scientific Director for Carcinogenesis:

Organizer of UICC-NCI Symposium on Carcinogens of Plant Origin, held in Bethesda, Maryland on April 8-10, 1968.

Vice-Chairman, Technical Advice and Review Committee on Biological Aspects of Air Pollution Problems (TR-8), Air Pollution Control Association.

Member, Editorial Advisory Board of Cancer Research.

Member, Editorial Board of Pesticide Monitoring Journal.

Member, Lung Cancer Task Force.

Vice-Chairman, Working Group on Less Hazardous Cigarette, Lung Cancer Task Force.

Advisor, Joint USDA-SAES Task Force on Tobacco.

Chairman, Chemical Carcinogenesis Contract Review Committee, NCI.

Member, Etiology Program Management Group, NCI.

Member, Cancer Chemotherapy National Service Center Contract Review Committee.

Participant in Conference on Chronic Disease Epidemiology, Rougemont, North Carolina, February 15-16, 1968, sponsored by DEHS.

Appointed representative of DHEW to the meeting of the Pesticide Residues Committee, Agricultural Research Institute, National Academy of Sciences, National Research Council, to discuss the significance of pesticide residues to human health, March 15, 1968.

Member, Joint FDA-PHS Study Group on Food Irradiation.

Member, UICC Committee on Exogenous and Endogenous Carcinogens.

Received from the Surgeon General, Public Health Service, the Superior Service Award, May 28, 1968.

CONTRACT NARRATIVES

OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR

FOR CARCINOGENESIS, ETIOLOGY

Fiscal Year 1968

AEC-NCI INTERAGENCY AGREEMENT, OAK RIDGE NATIONAL LABORATORY, (NCI-FS-64-13)

Title: Research in Co-Carcinogenesis

Contractor's Project Director: Dr. James L. Liverman

Project Officer (NCI): Dr. Carl G. Baker

Objectives: The broad objectives are: (1) to perform large-scale co-carcinogenesis studies in exposure chambers with controlled atmospheres containing selected chemical agents and/or viral agents with or without exposure to radiation in various combinations plus appropriate controls; and (2) to bring to bear on problems of carcinogenesis the exceptional capabilities of the ORNL Biology Division staff, especially in genetics and effects of radiation, and the ORNL facilities with analytical chemistry and engineering expertise, particularly in fabrication of instrumentation and special equipment. More specifically, there are five objectives: (1) to obtain bioassay assay data on cancer causation with multiple exposure to various agents by inhalation with and without radiation exposure; (2) to explore the transmission of genetic information with virus DNA and RNA as carriers for modifying cell function as measured by altered phenotypic expression, such as certain enzyme activity changes; (3) to determine enzyme, DNA, RNA and protein kinetic changes in chemical carcinogenesis; (4) to study chromosome alterations under various conditions of exposure to chemical, viral and radiation carcinogenetic agents; and (5) to obtain comparative data on mutagenic and carcinogenic responses in a variety of simpler organisms to different types of chemicals. Nearly half of the funding is associated with the first objective.

Major Findings: The large-scale, long-term inhalation experiments have just now reached the stage where the appearance of tumors can be expected in sizable numbers. The animals are living longer than anticipated in the original planning, and it will take additional time for final collection and analysis of data before additional new experiments can be planned and initiated as chamber capacity becomes available. Extensive computerization of the large amount of animal data continues to show its value. Present experiments involve long-term exposure of mice to chromium oxide dusts, ozonized gasoline fractions, myxovirus and radiation in various combinations. A large variety of the studies are conducted as part of the ORNL-NCI co-operative agreement by several groups at Oak Ridge. Selected findings from some of these studies are outlined.

The incidence of myeloid leukemia in germfree RFM males exposed to 300 r was only 1% in germfree animals compared to 13% in their conventionally reared counterparts. Similar incidence figures were obtained in females. In related experiments with methylcholanthrene feeding of RFM mice plus croton-oil-benzene painting, similar incidences in germfree animals (10.5%) were found compared to irradiated animals with conventional rearing (13%), but higher than in irradiated germfree animals (1%). It was postulated that the granulocytopoiesis induced by croton-oil-benzene painting may account for the increased incidences in the germfree animals.

In germfree animals the radiomimetic agent, triethylene melamine (TEM), resembles x-rays in certain somatic effects on RF mice. Studies have been conducted on the combined effects of TEM and x-rays administered concurrently. The incidence of thymic lymphomas with both agents exceeded that induced by either agent alone, and the dose-incidence curve showed less tendency to plateau at high dose levels than it did with either agent alone. The results suggest that the combined leukemogenic effects of the two agents were more additive than the combined cytotoxic effects.

In contrast to the failure to induce myeloid leukemia in RF mice with radiomimetic chemicals, the combined administration of methylcholanthrene and croton oil causes a high incidence (50%) of myeloid leukemia in a Japanese subline of RF mice. A study of the Oak Ridge subline of RF mice did not lead to increased incidences of myeloid leukemia upon treatment with methylcholanthrene; however, thymic lymphomas were induced in large numbers.

RF mice of 8 to 10 weeks of age were treated to dimethylnitrosamine (DMN) in drinking water continually for their life span. Cumulative tumor incidences of dying mice were: liver, 98%; lung, 96%; and stomach, 2.1% as compared with control values of lung, 20%; and liver and stomach, less than 1%. In studies with diethylnitrosamine (DEN) in BALB/c mice, mice dying after 17 weeks of treatment had a high incidence of tumors: liver, 21%; stomach, 52%; lung, 3%; esophagus, 6%. Vaccinated groups with either Rauscher leukemia virus or control vaccines showed no differences in tumor incidences between the two groups.

In a large experiment with 8 week old RF mice receiving whole-body exposure of 50-400 rad doses of 60-Mev protons or with 300-kvp x-rays, comparisons were made with another large group of mice exposed at higher radiation dose levels. Mean survival time of mice dying within the first month after the LD 50/30 was the same for both radiations, contrary to previous observations implying a higher RBE for protons at shorter survival times. Injection of AET 15 minutes before exposure gave a protection factor of about 2, raising the LD 50/30 for protons or x-rays to about 1200 rads.

Methods have been developed utilizing DEAE-cellulose chromatography for separation of aminoacyl-tRNA synthetases and tRNA's. The enzyme preparation from rat liver was active with at least 15 amino acids tested. Under standard conditions, the amino acid acceptor activities of normal and tumor (ethionine-induced hepatomas) tRNA's for several amino acids are very close. The elution profile of C^{14}-leucyl-tRNA from hepatoma shows five, and possibly six, peaks and appears very different from the normal. The tumor system is not sensitive to the small changes of magnesium concentration that inhibit the liver system. With either normal or tumor enzyme and at different enzyme levels, normal tRNA was charged better than tumor tRNA with C^{14}-leucine. Also, tRNA from hepatoma was aminoacylated at a decreased velocity. The results suggest that it is unlikely that the decreased velocity was due to an inhibitor present in the tumor-tRNA preparation. In summary, various changes occur in hepatomas by the feeding of ethionine at the tRNA level and at the synthetase level. Subtle tRNA changes occur which lead to decreased affinity for the enzyme even in the absence of gross changes of the chromatographic profile of the aminoacyl-tRNA.

Studies have been initiated on the biochemical pathways for different levels of protein synthesis with transplantable mouse plasma cell tumors, which are known to produce individual homogeneous myeloma proteins in different cell lines. Compared to organisms of lower phyla, mammalian tissue has a more complicated multiplicity of isoaccepting tRNA's. Preliminary results show that MPC62 and MPC47 tumor tissues have very different patterns of seryl-tRNA's. This suggests that the same kind of tissue performing different functions of protein synthesis may show difference in the quantity and quality of isoaccepting tRNA.

A new peak(s) of leucine-tRNA is observed when leucyl-tRNA from T2-infected cells is chromatographed. The new peak(s) is not present in normal cells and is only observed during the later stages of the infection cycle. Protein synthesis during the first 6 minutes of infection is required for formation of the new peaks. The new peak(s) appears on schedule even when phage DNA synthesis is inhibited with FUdR. A similar change is observed with phage T4. Results with S^{35} labeling suggest that de novo synthesis and/or thiolation is involved in the change.

A short-term effect of ethionine in rats is the rapid labeling of liver tRNA in vivo which may lead to the inactivation or changes in the coding properties of specific tRNA molecules. These changes have been related to the long-term effect of ethionine, including the development of hepatomas. Approximately one-half of the labeling is due to the ethylation of purines and there are at least five purine derivatives. The other half is found in the pyrimidine nucleotides, separable into two groups, likely the ethylated cytidylic acids and the ethylated uridylic acids. Also, the D-ribose moiety appears to be labeled. The synthesis of authentic ethyl derivatives of the purine, pyrimidine, and D-ribose moieties is proceeding in order to make positive identification.

Ethionine-ethyl-1-C^{14} will label the nucleic acids of rat liver in vivo, and 95% of the radioactivity is associated with the tRNA fraction. No labeling of DNA has been observed. This activity is located mainly in the liver, and nucleic acid synthesis is required for this incorporation to proceed. The major pathway for this ethylation of tRNA is not a random substitution for methionine, but a unique pathway which does not have S-adenosyl ethionine as an intermediate. The profile of tRNA's was markedly different from that obtained with methionine-$C^{14}H_3$. The activity of the preparation from ethionine-fed rats was decreased from 10 to 40% for half of the amino acids tested, suggesting that the ethylation has inactivated certain tRNA's. Of the amino acids tested no change was seen with methionine, glycine, tyrosine, threonine, and methionine. The ethionine-fed preparation had small new peaks with phenylalanine, leucine, lysine, and alanine, and larger quantitative shifts with proline, histidine, and tryptophan.

In studies of increased enzyme synthesis initiated by hormones, evidence obtained suggests that the steroid hormone acts directly, probably at the level of DNA-directed RNA synthesis to promote the synthesis of new RNA species required for enzyme synthesis. The protein hormones appear to act indirectly, via a hepatic intermediate which is limited in amount. Future studies on the nature of this intermediate are under way.

Growth hormone (STH) brings about a selective repression of the synthesis of hepatic tyrosine transaminase. STH acts secondarily to bring about this repression, probably by an initial effect on some extrahepatic (endocrine?) organ which in turn secretes the factor (hormone?) active in repressing enzyme synthesis. These studies on isolation and identificaion of the factor are continuing.

Turnover rates of soluble liver proteins are variable; differences as great as 30-fold are known to exist in the rates of degradation of functionally similar, soluble enzymes. With a combination of immunochemical and isotopic techniques, the turnover rate of rat liver tyrosine transaminase was found to be (t 1/2) 1.5 ± 0.3 hr. The half-life of the bulk of the soluble liver proteins is about three days. When protein synthesis was stopped *in vivo* by puromycin or cycloheximide, degradation of the enzyme (as well as its synthesis) virtually ceased. Additional studies on alternative hypotheses will be made.

The rat liver enzyme tyrosine-α-ketoglutarate transaminase (TKT) is not affected by adrenal steroids during gestation although it is readily induced by these agents in the adult. Fetal rat liver was studied in organ culture in a chemically defined minimal medium. Various additions can be made to the liver explants in an environment free of extrahepatic influence. The addition of hydrocortisone to such explants of fetal liver reproducibly induces tyrosine transaminase. The increased enzyme activity, usually five-fold within 5 hr., remained high unless steroid was removed, and then activity returned to the control level. Both actinomycin D and cycloheximide blocked the induction by hydrocortisone. Immunochemical techniques demonstrated that the increase in enzyme activity was due to the *de novo* synthesis of enzyme protein.

Studies have been performed to determine if hydrocortisone, insulin, or glucagon regulated TKT synthesis by a direct influence on hepatic cells. By utilizing isolated livers from adrenalectomized rats perfused by the technique of Miller *et al*., hydrocortisone elicited a sharp increase in TKT activity, the enzyme level increasing linearly for the duration of liver perfusion (up to 11 hr.). Insulin and glucagon also increased the activity of TKT in perfused livers. Growth hormone did not affect TKT activity when added directly to the perfusion medium. However, blood from growth-hormone-treated rats lowered the level of TKT activity when perfused into livers isolated from untreated rats. Thus, the results indicate that hydrocortisone, insulin, and glucagon induce TKT by direct action in the liver whereas the repression observed after growth hormone treatment in vivo apparently occurs indirectly.

Hydrocortisone, a hormonal inducer of tyrosine transaminase in rat liver, has been found to induce this enzyme in cultures of the minimal deviation Reuber hepatoma. The hydrocortisone response was maximal by 12 hr., and transaminase activity remained at this level as long as 24 hours. If induced cells were placed in fresh steroid-free medium, the enzyme rapidly declined toward basal levels with a half-life of 4 to 6 hr. Although total RNA synthesis was little changed by hydrocortisone, actinomycin D blocked the hormonal induction of the enzyme.

The first x-ray crystal structure determination of a pyrimidine photodimer has been accomplished. The results provide (1) the conclusive demonstration of the cyclobutane ring structure for the dimer and (2) structural details of the molecule. The dimer molecules are centrosymmetric and the data correspond only with isomer IV (trans-anti configuration). Three-dimentional x-ray data were collected on the Oak Ridge Computer-Controlled Diffractometer. The structure was solved by direct methods, with the use of the computer program "MAGIC".

Other physicobiochemical studies on protein structure are under way. Heavy atoms are being introduced into proteins to obtain derivatives suitable for x-ray crystallographic analysis. Specific labeling of active sites of enzymes is also being accomplished by employing reagents that possess certain structural features of the natural substrate. Rubredoxin, an electron carrier derived from Cl. pasteurianum 2nd containing nonheme iron, was chosen as a model protein for x-ray structure analysis because of its low molecular weight, stability to various denaturing agents, crystallinity, and suitable amino acid composition. Preliminary experiments indicate that the N-terminal amino group may be reacted preferentially and that no gross changes in tertiary structure accompany the modification. Other studies of protein structure employing physical methods on model systems, polypeptides, and proteins are being conducted.

Work with x-rays and TEM led to the conclusion that at least some of the mutations induced by these agents arise from premutational damage that is not fixed as final mutation until DNA synthesis occurs and is subject to elimination, probably by repair, before fixation. The evidence for this is of two kinds: (1) The mutation yield can be altered by postmutagen treatment with streptomycin when this treatment is begun before DNA synthesis (S period) but not after DNA synthesis. (2) The mutation yield is decreased the longer the interval between the mutagenic treatment and DNA synthesis. Both kinds of evidence have now been obtained with 2537-A ultraviolet radiation (uv) and with N-methyl-N'-nitro-N-nitrosoguanidine (NG). The decrease in mutation yield as the time between mutagenic treatment and the S period increases was more rapid with uv and NG than with x-rays and TEM, suggesting a more rapid rate of repair. Streptomycin posttreatment usually causes a decreased mutation yield with x-rays, TEM, and uv, but an increased yield with NG. Occasionally, decreased yields have been found with NG and increased yields with x-rays. The decrease in yield has been interpreted as the result of a block in the onset of the S period with consequent increased time for repair. Preliminary evidence suggests that the increased yield may result when the S period is not completely blocked, but occurs during the period of streptomycin exposure. These studies were conducted in paramecia.

Cell line V79-129D1 of the Japanese hamster was selected as a model system for somatic cell kinetics because of its relatively simple ($2n = 22$) and stable karyotype at a near-diploid level, active growth, high plating efficiency, and its adaptability to growth in a defined medium. A number of clones have been recovered which, unlike the parental cells, are no longer capable of utilizing L-glutamic acid but require L-glutamine for growth. This change is heritable and revertible. Results indicate that reverse mutations occur randomly and are estimated to be 2.3×10^{-8} per cell per generation. The cells are sensitive to 8-azaguanine at concentrations greater than 0.1 µg/ml. By mass selection, a cell subline was derived which exhibited a 1000-fold increase in resistance to the drug as compared to the parental cells. The data support the conclusion that resistance is due to a randomly occurring spontaneous mutational event and not to adaptation. The reversion rate to drug sensitivity was found to be 10^{-7} per cell per generation. Somatic cell hybrids were produced through fusion by plating together the glutamine-requiring cells and azaguanine-resistant cells in a medium in which neither of the parental cell types could survive. This tetraploid hybrid clone can be propagated through apparently normal mitosis. Functional tests indicate that both glutamine auxotrophy and azaguanine resistance behave as recessive characters in the hybrid cell.

The Shope papilloma virus induces a virus-specific arginase in rabbit squamous cell epithelium. A lowered blood arginine is a systemic effect associated with such infection, which induces papillomas in the skin. Blood arginine levels can be used to indicate whether human subjects have worked with the virus. About half the individuals having had contact with the virus could be separated out (blind) from normals through changes in their blood arginine levels. Comparison of arginase induced in the rabbit by a wild-type virus and by a mutant virus indicates that a number of physicochemical properties differ between the two arginases, including a wide separation on carboxymethyl cellulose chromatography. It is suggested that this structural difference is related to differences in the original monomers modifying either the formation or stability of the polymer. The results suggest that the enzyme is derived from virus information rather than from rabbit information and that the mutation of the virus is directly associated with the change in structure of the enzyme.

It is theoretically possible for coded information to be introduced into an organism with virus as a vector; this information may be innately part of the virus, that specifically transduced from other genomes, or that synthesized in the laboratory and attached to the virus DNA or RNA. Tobacco mosaic virus RNA was modified and used in studies with poly-A-RNA-infected plants along with control normal TMV-RNA-infected plants. Tryptic digestions were performed on basic proteins derived from the two types of plants. Products were characterized by chromatography. Lysine containing peptides which cochromatograph with mono-, di, tri, tetra, and pentalysine have been found in the material from the poly-A-RNA infected plants. The control material yielded lysine containing peptides only in the monomer position and a trace amount which moved like trilysine.

Although photoxidation of protein in vitro is known to involve a preferential photodamage to the histidine moieties, preferential attack of protein histidine in vivo has not been confirmed. Assay of preferential histidine photosensitization was studied with *E. coli* and benzo(a)pyrene in comparison with methylene blue as photosensitizer. By two assay methods preferential photosensitization occurred with both compounds. The illumination dose required to detect histidine loss is several times greater than that required to cause 99% kill. Simplified assay methods were also developed utilizing tritiated histidine.

From early studies on the recovery potential of immunologically competent progenitor cells from x-irradiation, it has been possible to show that the compartment of immunologically uncommitted progenitor (PC_1), that is, the primary antibody-forming potential, has the capacity to recover from an x-ray insult. In contrast, the compartment of immunologically committed progenitor cells (PC_2), that is, the secondary antibody-forming potential, lacks the capacity to recover from x-irradiation. Some effect on the secondary response recovery could be produced, however, by altering the amount of antigen given as primary injection or the time interval between priming and x-irradiation. If antigen persisted from the primary injection beyond the time of irradiation, the PC_2 cell pool recovered partially and in some instances even to near preirradiation levels. The results suggest that cells from the PC_1 cell pool (which was shown to regenerate after x-irradiation) are transformed by persisting antigen into PC_2 cells. Thus, the recovery of the PC_2 pool from x-irradiation is dependent on: (1) persistence of antigen; and (2) presence of PC_1 cells, and therefore appears to be a manifestation of the regenerative potential of the PC_1 cell compartment.

Recent studies have demonstrated that the phagocytic and degradative capacitites of peritoneal phagocytes are resistant to supralethal levels of x-irradiation. I^{125}- labeled human gamma globulin (HGG) was employed as test antigen and mice received whole-body-irradiation from 0 to 600 r either immediately before injection with labeled antigen or 10 days after. In a third group, x-rays were given prior to an injection of unlabeled HGG, and labeled HGG was injected 20 days later. Data from these experiments are summarized as follows: X-irradiation up to 600 r did not interfere with trapping of labeled HGG in germinal centers given in a primary injection; however, it caused enhanced clearance of the labeled antigen from the germinal centers regardless of whether x-rays were given before or after the test antigen. When x-rays were followed immediately by a primary injection of unlabeled HGG, then the initial trapping of a secondary injection of labeled test antigen given on day 20 after x-ray was less efficient than that of unirradiated controls, and elimination of labeled antigen from the germinal center was enhanced. The data show that x-irradiation, besides affecting the immune progenitor cell pool also affects persistence of antigen (and under certain conditions efficiency of the trapping mechanism) in the germinal centers of the lymphatic tissues.

Investigations were performed on the effect of various macroglobulin preparations (19S) on radiation survival and recovery in mice. Isologous macroglobulin caused a significant increase in survival after 750 r irradiation. Comparable results were obtained with rat macroglubulin. Results were interpreted to show that an active material is an intrinsic serum constituent associated with 19S protein of mice and rats and functions across species barriers. The activity of this fraction is associated with enhanced radiation survival and recovery of proliferative potential of the hematopoietic stem cell pool.

Electron microscopic autoradiography was used in a systematic study of the first 30 days of the primary immune reaction in mice to I^{125}-labeled human gamma globulin. During the first 24 hours of the primary response, the label was detected in phagocytic cells of the marginal zone (macrophages) and in the periphery of the germinal centers (reticular cells). From day 4 on, antigen was clearly retained extracellularly in the membranous infoldings of reticular cells in the germinal centers. The spongelike nature of these antigen-retaining reticular cells enables the pseudopodia of immunoblasts to come in firm contact with the antigen, as seen in some of the electron micrographs. Villous extensions of immunoblasts surrounding the antigen-retaining processes of the reticular cells were observed to be in intimate contact with the labeled electron-dense material, occasionally seen intermixed with ribosomes. By the end of the fourth week, there was no detectable grains present in germinal centers, and the amount of plasma membrane infoldings was minimal. A large number of virus-like particles was observed in the germinal centers, compared to the associated lymphocyte mass of primary nodules in the spleen. In the centers the particles were primarily located extracellularly in the infoldings of antigen-retaining reticular cells. Occasionally, virus-like particles were also observed budding from typical immunoblasts.

These major findings constitute several highlights from the extensive work at Oak Ridge. Other studies and further details can be found in the Progress Reports of the Oak Ridge National Laboratory.

Significance to Biomedical Research and the Program of the Institute:

Bioassay and basic research on the mechanism of action of environmental carcinogenic agents in eliciting the development of cancer in experimental models is of great significance as it looks into combined effects of three important types of carcinogens: chemical, viral and ionizing radiation. Results of these experiments may permit correlation of laboratory findings with the appearance of neoplasms in man. The support for this work at Oak Ridge National Laboratory is in the form of broad support to the total operations of the Oak Ridge National Laboratory drawing in the special skills which can be brought to bear on problems of cancer significance. Emphasis on radiological aspects of cancer causation has been relatively low within most programs within NCI because of the extensive capability and interest within the AEC programs. Therefore, it is of considerable importance to insure sizable efforts in radiological aspects of cancer causation which this project accomplishes.

Proposed Course of Project: Continuation of the program aimed at attaining the objectives described above; however, if budget cuts are imposed, the size of this project will be reduced accordingly.

Date Contract Initiated: January 14, 1963

Current Annual Level: $2,000,000

UNIVERSITY OF NEBRASKA (PH43-68-959) -- (Formerly CHICAGO MEDICAL SCHOOL PH43-65-67)

Title: A Resource for Carcinogen Bioassay and Related Carcinogenesis Research

Contractor's Project Director: Dr. Philippe Shubik

Project Officer (NCI): Dr. Carl G. Baker

Objectives: The purpose of this contract is two-fold in nature: the bioassay of agents suspected of being carcinogenic, and fundamental research on mechanisms of carcinogenesis.

(1) Bioassay: Compounds are selected for carcinogenic screening on the basis of several criteria, including (a) suspicion as a result of epidemiological studies uncovering suspected high risk to cancer in defined environmental exposures; (b) chemical properties and structures or physical properties suggesting a high index of suspicion for potential carcinogenic potency, and (c) potential or actual universal distribution or use of agents suggesting the need for carcinogenic bioassay. The techniques of bioassay include administration by various routes. Selection of various modes of administration is determined by properties of the materials, comparative response in various species, determination of dose response parameters, and the desire for maximum extrapolation to man. Protocols for these experiments take into account the current state of knowledge in the field as well as the properties of the agents being tested. Experimental design also utilizes the skills of the Biometry Branch and other disciplines so that data analysis and interpretation become part of the protocols. A large number of compounds are under bioassay, using several dose levels and multiple species.

(2) Research on the mechanism of carcinogenesis: Objectives include fundamental research at the cellular and host levels for the purpose of elucidating biological mechanisms involved in the carcinogenic process and to identify the factors controlling or modifying the natural history of induced neoplasms. Methods employed include the determinations of anatomic and metabolic fates of environmental carcinogens to determine sites of elective localization and paths of detoxification and excretion. Identification of the active form of the carcinogen, characterization of the enzymatic, immune or other host factors critical to the carcinogenic responses, and comparison of response in different species are integral parts of this program.

Major Findings: The following list of compounds have been under test in mice, rats and hamsters, in adults and newborns, by various routes of administration. Of those studies that are finished, most of the compounds have been negative for carcinogenesis. In addition to those on test, the following four compounds are planned for evaluation as capacity becomes available: Metronidazole, Phenazopyridine, Diatrizoate, and Meclinizine. Compounds on test the past year are:

Ethoxazene
Sulfisoxazole
Sulfamethoxazole
Methocarbamol
Alpha-Naphthylamine
o-Dianisidine
o-Tolidine
3,3'-Dichlorobenzidine
DDT
Carbon Tetrachloride
Chloroform
1,1,1-Trichloroethane
1,1,2,2-Tetrachloroethane
Trichloroethylene
Tetrachloroethylene
Isonicotinic Acid Hydrazide
Isonicotinic Acid
Acetylsalicylic Acid
Hydrazine Sulfate
Antabuse
Ethinamate
Phenylbutazone
Thorazine
Banthine
Miltown
Diabinese
Fulvicin
Librium
Phenacetin
Vioform
Daraprim
Nardil

Selected examples of research projects from the nontesting portion of the project include the following: As part of the continuing examination of the relationship between structure and tumorigenic activity among the nitrosamines, some new cyclic nitrosamines have been prepared and are under test. The purpose of the studies is to attempt to ascertain the mechanism by which cyclic N-nitrosamines form alkylating agents. Increasing size of the molecule does not appear to diminish the carcinogenic activity, as is the case with nitrosamines derived from open chain secondary amines. Preliminary information suggests no correlation between acute toxicity and carcinogenicity. Two of the latest compounds are nitrosoheptamethyleneimine and nitrosooctamethyleneimine. In addition, 1-nitrosopiperazine has been prepared and is under test.

Rats treated with a high dose of dimethylnitrosamine (DMN) incurred severe liver damage, followed by very active liver regeneration, similar to that seen with partial hepatectomy. A high incidence of kidney tumors but not liver tumors was found at high dose levels. Differences in methylation of RNA and DNA at different stages of regeneration after partial hepatectomy are being sought. Incorporation of radioactivity into the DNA was highest at 72 hours. Another group of animals is being fed DMN following hepatectomy. With chronic DMN feeding, changes in base composition of liver RNA and DNA and possibly changes in quantitative methylation of these nucleic acids is also under study. Nitrosamines are known to produce gastrointestinal, oral and pharyngeal tumors in rats and mice, but less information on skin carcinogenesis is available. The following five compounds are being studied for skin carcinogenesis: (1) Nitrosohexamethylene imine, (2) Nitrosomethyl cyclohexylamine, (3) Dinitrosopiperazine, (4) Dimethylnitrosamine, and (5) Nitroso-Dicyclohexylamine.

Significance to Biomedical Research and the Program of the Institute:

Dr. Shubik and his group have consistently produced major research results in chemical carcinogenesis in high output. He has actually provided, therefore, considerable training in a field not very popular with many investigators, in part because of the long-term nature of many of the experiments, sometimes with negative results. With the move to the University of Nebraska graduate students will be an added feature to the contract. With the shortage of centers conducting broad programs in chemical carcinogenesis, it was determined important to continue with the contract despite the difficulties related to moving the project from the Chicago Medical School.

Proposed Course of Project: It is anticipated that the project will continue along the same lines as at the Chicago Medical School with the addition of graduate students to the program. Care was taken in planning for the move to insure that a minimum number of animals would have to be moved, and the studies will be increased as rapidly as possible at the new site. It is further planned that the costing arrangements initiated at the Chicago Medical School will be reinstituted at the University of Nebraska (i.e., the amount available in the contract for studies in the "basic" part of the project will not exceed the amount expended for bioassay and testing activities). It will take considerable time to reinstitute this system, but this balance must be attained no later than 15 months after the initiation of the contract at the University of Nebraska. The amount next year is decreased from about $1 million to $750,000.

Date Contract Initiated: University of Nebraska -- March 18, 1968

Chicago Medical School -- December 1, 1964

Current Annual Level: $750,000

CONTRACT NARRATIVES
OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR
FOR CARCINOGENESIS, ETIOLOGY
Fiscal Year 1968

FOOD AND DRUG RESEARCH LABORATORIES (PH43-64-933)

Title: Studies on Mucous Flow in the Intact Mammal Exposed to Tobacco Smoke

Contractor's Project Director: Dr. Steven Carson

Project Officer (NCI): Dr. Hans L. Falk

Objectives: To measure changes in physico-chemical properties of mucus in the tracheobronchial tree of the cat inhaling cigarette smoke; to compare these changes with ciliary efficiency in moving the mucous sheath; to study the penetration of cigarette smoke aerosol particles through the mucus.

Major Findings: Important observations were made regarding viscosity of mucus and the importance of secondary factors was discovered which interfered with direct corelation of the changes in viscosity with cigarette smoke exposure. The implantation of bioelectrodes has been perfected with minimal trauma and smoke administration and recording of results has been automated. Changes in electrical characteristics of the mucus have been observed on exposure to cigarette smoke and are being compiled to complete the study.

Significance to NCI Program & Bio-Medical Research: These studies may well serve as one of the base lines for future exploration of cigarette smoke effects on biological constituents of the tracheobronchial tree in the quest for a less hazardous cigarette under the leadership of the Lung Cancer Task Force.

Proposed Course of Project: The research project has been given extra time without additional cost to complete the study in a few months.

Date Contract Initiated: June 22, 1964

Current Annual Level: June 22, 1966 - September 20, 1968: $153,000
Funded in FY 1966--no additional funds for FY 1968.

UNIVERSITY OF SAN FRANCISCO (PH43-64-886)

Title: Study of Carcinogenicity of Hydrocarbon-Metal Complexes

Contractor's Project Director: Dr. Arthur Furst

Project Officer (NCI): Dr. Hans L. Falk

Objectives: To study the carcinogenic potential of certain industrially significant heavy metal compounds and complexes, particularly those of nickel, cobalt, iron, titanium and others.

Major Findings: On intramuscular injection of these compounds in a lipid vehicle, sarcomas have been produced at the site of injection with all nickel compounds under test. Tumors have also been observed with titanocene, but only one was induced with titanium oxide. Ferrocene was inactive.

Because of the high carcinogenicity of nickel compounds, the rate of elimination of nickel has been under study starting with different nickel complexes. The major portion is excreted in a few days.

Significance to NCI Program & Bio-Medical Research: These organo-metallics are of considerable industrial significance and in certain cases promise to become general air pollutants.

Proposed Course of Project: These studies will be continued to explore further the risks involved in the widespread use of organic and inorganic complexes of certain metals.

Date Contract Initiated: June 3, 1964

Current Annual Level: December 31, 1967 - December 2, 1968: $48,125

HAZLETON LABORATORIES, INC. (PH43-64-1161)

Title: Study of Multiple Factors in Carcinogenesis: Infarction, Metal Dusts and Hydrocarbon Inhalation

Contractor's Project Director: Dr. H. N. MacFarland

Project Officer (NCI): Dr. Hans L. Falk

Objectives: To determine the effects of pulmonary infarction, induced by intravenous injection of fluorocarbon into rats, on lung cancer incidence from inhalation of cigarette smoke, nickel sulfide or ozonized gasoline.

Major Findings: Excellent survival was obtained in the rats which have all been sacrificed by now. No results are as yet available.

Significance to NCI Program & Bio-Medical Research: Inhalation of dusts and aerosols in order to explore the factors necessary for the production of epidermoid carcinoma of the lung had focused attention on the findings of Stanton and Blackwell, which were to be extended and confirmed by this study. The study of combined, synergistic or antagonistic effects in the field of lung cancer induction is a very important problem.

Proposed Course of Project: All animals have been sacrificed and histopathologic evaluation is nearly complete. Final report will be available in a few months.

Date Contract Initiated: June 29, 1964

Current Annual Level: October 1, 1966 - June 30, 1968: $91,138
Funded in FY 1967--no additional funds for FY 1968.

GENERAL FOODS CORPORATION (PH43-63-1165)

Title: Oxidation Products of Fats

Contractor's Project Director: Dr. R. J. Sims

Project Officer (NCI): Dr. Hans L. Falk

Objectives: Epoxides, peroxides, hydroperoxides, lactones, polymers, and other products result from the oxidation of fats in the process of food preparation and storage. Since several of the above mentioned groups of chemicals have carcinogenic properties, these compounds, their mode of formation and their carcinogenic properties are in need of study. Once the major oxidation products are identified, their biological activity can be established in feeding experiments.

Major Findings: A number of commercial fats have been heated under controlled conditions and the formation of oxidation products have been followed. Methodologic improvement was required before epoxides could be determined quantitatively. Identification of the epoxides in these heated fats has been completed. A series of lactones have also been identified in heated fats. The formation of epoxycholesterol has been studied. No polycyclic aromatic hydrocarbons have been detected under any conditions of heating utilized in these studies.

Biological studies have been undertaken to determine any potential carcinogenic hazard from eating epoxy fatty acids or epoxycholesterol. The former compounds were not acutely toxic as was the latter, but produced in the animals on this diet very high serum cholesterol levels and cardiovascular and cerebrovascular accidents. These findings are communicated to scientists at the National Heart Institute. The long term studies have not advanced far enough to produce cancer.

Significance to NCI Program & Bio-Medical Research: Some components of the groups of chemicals found in heated fats are suspect of carcinogenic activity. Several feeding studies carried out by other investigators have confirmed the carcinogenic activity on feeding or injection of certain fat fractions, but no chemical identification has been made. In these studies pure specific oxidation products of fats are fed to rats.

Proposed Course of Project: The final feeding studies are under way and so are studies to follow the epoxycholesterol which so far could not be accounted for either in the feces or in body fats. In 18 to 24 months these studies should be ended.

Date Contract Initiated: June 18, 1963

Current Annual Level: June 18, 1967 - June 17, 1968: $106,279

UNIVERSITY OF WISCONSIN (PH43-66-888)

Title: Carcinogenic Activity of Bacteriostatic Nitrofurans and Related Chemicals

Contractor's Project Director: Dr. George T. Bryan and Dr. J. M. Price

Project Officer (NCI): Dr. Hans L. Falk

Objectives: To determine the carcinogenic hazard of bacteriostatic drugs belonging to the nitrofuran series; to correlate chemical structure with carcinogenic activity and target specificity.

Major Findings: Because 2-(2-formylhydrazino)-4-(5-nitro-2-furyl)-thiazole turned out to be a potent carcinogen for mammary gland, kidney, and intestine, while 5-nitro-2-furaldehyde semicarbazone only produced benign mammary lesions and N-(5-nitro-2-furfurylidene)-1-aminohydantoin was not carcinogenic at all, a series of related compounds has been tested for carcinogenic activity.

2-N-formylamino-4-(5-nitro-2-furyl)-thiazole turned out to be the most interesting drug because, besides benign mammary tumors and a few renal carcinomas, it induced bladder cancer in 100% of the animals. Recently, it was found that replacement of the formyl group by an acetyl group abolished the carcinogenic activity as can be judged thus far.

Significance to NCI Program & Bio-Medical Research: Minor structural changes in the nitrofuran derivatives produced considerable variation in carcinogenic potency and target orientation. Several of these compounds are in use as bacteriostatic agents, while others are used as feed additives. Feeding these drugs and related compounds to female rats has enlarged our understanding of the carcinogenicity of nitrofuran compounds as well as aromatic amines and stressed the chemical structure as very important variables for kidney, intestine, bladder and mammary glands as target organs for cancer development.

Proposed Course of Project: The contract will be terminated at the end of the third bioassay experiment.

Date Contract Initiated: June 10, 1966

Current Annual Level: June 10, 1967 - June 9, 1968: $30,000
Funded in FY 1967--no additional funds for FY 1968.

COLUMBIA UNIVERSITY (PH43-67-731)

Title: Carcinogenicity of Fresh and Mildly Oxidized Vegetable and Animal Fats

Contractor's Project Director: Dr. H. Kaunitz

Project Officer (NCI): Dr. Hans L. Falk

Objectives: To determine in controlled feeding experiments in rats the potential carcinogenic hazards of dietary fats under conditions of common use.

Major Findings: This contract has been in existence less than one year and no results are as yet forthcoming.

Significance to NCI Program & Bio-Medical Research: Experiments have been under way for several years at different universities to ascertain the biological and nutritional value of different fats in the diet. Most of these studies concluded that there was an inexplicable increase in tumors at various sites in many of the animals on the vegetable fat diet compared to other diets. None of the investigators could explain the difference on the basis of longevity, weight gain or intercurrent events unrelated to the diet. Since the fats involved in those studies were the ones most frequently used in man's diet, it was thought necessary to further explore the problem and to include the study of the carcinogenic potential of irradiated fats.

Proposed Course of Project: The program will last at least three years, since it involves feeding rats throughout their normal life span and analyzing the data.

Date Contract Initiated: June 15, 1967

Current Annual Level: June 15, 1967 - June 14, 1968: $85,000

UNIVERSITY OF CINCINNATI (KETTERING LABORATORIES) (PH43-64-45)

Title: The Co-Carcinogenicity of Aliphatic Hydrocarbons and Organic Disulfides

Contractor's Project Director: Dr. E. Bingham Mattheis

Project Officer (NCI): Dr. Hans L. Falk

Objectives: To determine the carcinogenicity or co-carcinogenicity of hydrocarbons, sulfur-containing compounds or organic chemicals of industrial significance and to identify the chemicals in their role as contributors towards carcinogenesis; to delineate the structural requirements for co-carcinogenic potency of paraffins, olefins, alkyl benzenes, fatty alchols, esters, etc.; to determine carcinogenic or co-carcinogenic properties of ingredients of incomplete Freund's adjuvant and related compounds.

Major Findings: Extremely low concentrations of benzo(a)pyrene were found to be carcinogenic for the skin of mice when dissolved in a solvent such as n-dodecane, while the concentration of the same carcinogen had to be much higher with decahydronaphthalene as solvent. Additional experiments pointed to alkanes and alkenes of straight chain length with 12 carbons as the most active co-carcinogens with steady decrease of activity as the chain length was increased or decreased. Similar results were obtained for straight chain fatty alcohol as co-carcinogens. Elemental sulfur and several polysulfides were also co-carcinogens, but not carcinogenic. Incomplete adjuvant and individual components were studied for carcinogenic and co-carcinogenic activity. Although none showed co-carcinogenic activity in the system under study, the incomplete adjuvant and specifically Drakeol 6 on intramuscular injection produced numerous granulomatous lesions away from the site of injection to require further study. Among other industrial chemicals studied on this contract were urethane formaldehyde complexes, which did not produce tumors but damaged the adrenals.

Co-carcinogenic studies were extended to use other initiators than benzpyrene, which gave similar but not as drastic results when their concentration was reduced in the presence of promoting compounds. Variation in the quantity of promoting chemical was also studied and it was found that as the proportion of promoting chemical decreased the co-carcinogenic effect was abolished.

Significance to Bio-Medical Research and the Program of the Institute: The carcinogenicity of polycyclic aromatic hydrocarbons can be modified by the presence of co- or anticarcinogenic chemicals. In man's environment the carcinogen is usually encountered associated with other related as well as unrelated chemicals and the evaluation of the carcinogenic hazard without information on these associated chemicals is difficult and often impossible. These findings are of great importance towards an understanding of the effects of low concentrations of polycyclic hydrocarbons. Cigarette smoke and community air pollution contains small amounts of these chemicals and the deliberate introduction of anticarcinogenic agents may ultimately reduce the hazard. There is need for this information to determine whether safe concentrations of these polycyclic hydrocarbons in man's environment may exist.

Proposed Course of Project: Current animal experiments will be brought to an end while recently designed studies will be initiated.

Date Contract Initiated: September 11, 1963

Current Annual Level: October 1, 1967 - September 30, 1968: $34,062

WYETH LABORATORIES, INC. (PH43-65-16)

Title: A Quantitative Evaluation of the Immunological Effect of Carcinogen, Co-carcinogen, and Anti-carcinogen

Contractor's Project Director: Dr. B. A. Rubin

Project Officers (NCI): Dr. T. Borsos, Dr. Hans Falk, Dr. Herbert Rapp

Objectives: To determine the relationship between the tumorigenic and the tolerance-inducing properties of carcinogenic polycyclic hydrocarbons, and to evaluate the effect of known or potential co-carcinogenic and anti-carcinogenic polycyclic hydrocarbons in the parallel assay systems of tolerance and leukemia induction.

Major Findings: Following a successful coded study of polycyclic hydrocarbons of different potency by the tolerance induction test, a second set of compounds has been made available to the project director to run parallel studies on leukemia induction versus tolerance induction.

Earlier studies had also established that co- or anticarcinogenic compounds together with the polycyclic hydrocarbon carcinogen would enhance or decrease the tolerance induction. For certain compounds however the results were not as clear cut, but seemed to depend on the type of compound and the relative concentration of the compounds to each other in the leukemia induction test.

Significance to Bio-Medical Research and the Program of the Institute: This project is designed to investigate the basic mechanisms of carcinogenesis by polycyclic hydrocarbons, and to determine the role of host immunologic response in this process of carcinogenesis. Knowledge of the mechanism of carcinogenesis at the level of host cell-chemical interaction is fundamental to all forms of carcinogenesis research. On a more immediate practical level is the possibility of applying the tolerance induction system as a screening procedure for the rapid detection of potential polycyclic hydrocarbon carcinogens, co-carcinogens or anti-carcinogens, the type of chemicals encountered in tobacco smoke and community air pollution.

Proposed Course: Further study may be required because the chemicals used for the tolerance induction and for leukemogenesis are common air pollutants and cigarette smoke constituents.

Date Contract Initiated: September 14, 1965

Current Annual Level: September 14, 1967 - September 15, 1968: $82,793

CITY OF HOPE MEDICAL CENTER (PH43-65-1036)

Title: Mycotoxins in Foodstuffs

Contractor's Project Director: Dr. Riojun Kinosita

Project Officer (NCI): Dr. Hans L. Falk

Objectives: To investigate the carcinogenicity and toxicity of mycotoxins found in foods fermented with the help of fungi belonging to the *aspergilli* and *penicillium* species.

Major Findings: The toxicity of extracts of miso--a Japanese food consisting of soybeans, barley and rice fermented by specific molds belonging to the *A. oryzae-flavus group*--has been determined on mice and ducklings. Extracts obtained from the cultures of other *aspergilli* have been tested and were toxic to the liver or induced gastric lesions, particularly when given with a high dose of sodium chloride per os. The gastric lesions included epithelial necrosis, mucosal hemorrhages, erosion and epithelial proliferation. Particularly active were extracts of *A. versicolor*, *tamarii* and *flavus*. Besides studies on Japanese food, considerable efforts were made to isolate and grow the fungi present in the typical soya bread prepared by South Koreans and felt to be possibly responsible for gastric cancer, which is particularly frequently encountered in South Korea. Several toxic strains of fungi have been isolated so far. Additional studies were carried out on the fungal contaminants of certain common cheeses, but no toxic fungal metabolites were found. Several hundred food samples from Uganda have been obtained and are being studied for the presence of aflatoxins and other toxins.

The observations have been confirmed that fungi isolated from certain staples may not produce any mycotoxins when grown on certain media. The importance of multiple media for determination of toxin formation has been stressed.

Several test systems have been set up to determine mycotoxin activity with rodent, chick and bacterium as target species.

Significance to Bio-Medical Research and the Program of the Institute: Many population groups prefer their major dietary constituents to be fermented, often without starter cultures, so that toxic fungi may be present. This study therefore was extended beyond Japan and Korea to include the Philippines and Uganda with specific emphasis on area where a high incidence of gastric and liver cancer has been recorded. The food samples from Uganda have been identified as originating from high or low cancer incidence areas.

Proposed Course of Project: This project should continue for several years in the same direction. Identification of mycotoxins will be carried out by collaboration with Drs. Wogan and Buchi at Massachusetts Institute of Technology.

Date Contract Initiated: June 29, 1965

Current Annual Level: December 31, 1967 - December 30, 1968: $100,600

SOUTHWEST RESEARCH INSTITUTE (PH43-65-1000)

Title: Research on Free Radicals and Alkylating Agents in Tobacco Smoke

Contractor's Project Director: Dr. Donald E. Johnson

Project Officer (NCI): Dr. Hans L. Falk

Objective: To detect, characterize, and evaluate free radicals and alkylating agents in tobacco smoke and incense and to determine their carcinogenic properties.

Major Findings: Methods have been developed for the detection and identification of nitrosamines in cigarette smoke by means of gas chromatography and a specific sensitive nitrogen detector. The specific nitrosamines have not as yet been identified. High nitrate tobacco is being made available to the contractor by the U. S. Department of Agriculture to determine nitrosamine in the smoke. Several important observations have been made on low boiling constituents of cigarette smoke--all oxides of nitrogen--which give strong electron spin resonance signals when they react with hemoglobin or phospholipids in the lungs of rabbits. Phospholipids possessing serine or ethanolamine are affected. Very similar observations were made when incense smoke was used instead of cigarette smoke.

Bioassay on fresh instant smoke condensate, collected on a specially designed smoking machine, have been under way and should show by next FY whether condensate with the free radicals reduced or completely removed will give a lower tumor incidence on the back of mice.

Significance to NCI Program & Bio-Medical Research: The identification of free radicals and alkylating agents in tobacco smoke is of importance since these compounds may contribute to the lung cancer burden of the heavy cigarette smoker. When identification and quantitation have been achieved and bioassay experiments establish their contribution of carcinogenic activity of the condensate, steps can then be taken to remove these chemicals and free radicals.

Proposed Course of Project: The identification and characterization will be continued. The bioassay program is under way and must be completed before the contract is terminated.

Date Contract initiated: June 23, 1965

Current Annual Level: December 23, 1967 - December 22, 1968: $93,430

TEMPLE UNIVERSITY, FELS RESEARCH INSTITUTE (PH43-65-1029)

Title: Carcinogenic Dermatophytes

Contractor's Project Director: Drs. F. Blank and M. B. Shimkin
Project Officer (NCI): Dr. Hans L. Falk
Objectives: To determine whether dermatophytic fungi produce carcinogens, since several of them are known to produce fluorescent lactones.

Major Findings: Injection of certain fractions of extracts of the mycelium of Candida parapsilosis--a human parasitic fungus--produced sarcomas at the injection site of 9 of 36 animals. This activity was concentrated in the sodium carbonate extract, and further research is under way to identify the carcinogenic component. For less active extracts were obtained from other dermatophytes, which are also under further investigation. A search for other neoplasms in these animals is under way. Whether the compound responsible for the carcinogenic action is a lactone has thus far not been established. Pure compounds which have been isolated from these fungi did not produce any tumors. The nature of the active compounds has as yet not been established, but large scale production of mycelium and spent medium of Candida parapsilosis has been under way to allow isolation of the active compounds and their identification. Not only sarcomas were at the site of injection, but also the leukemia incidence was enhanced in certain groups of mice injected with these extracts.

Significance to NCI Program & Bio-Medical Research: This program deals with the possible existence of carcinogenic mycotoxins in man's immediate environment in addition to his exposure to mycotoxins in his food.

Proposed Course of Project: Future work will deal with potential mycotoxins in Candida albicans and possibly later on in alternaria species, a well-known metabolite producing fungus growing on tobacco and tomato among important products.

Date Contract Initiated: June 22, 1965

Current Annual Level: January 1, 1968 - December 31, 1968: $118,682

GENERAL FOODS CORPORATION (PH43-64-1158)

Title: A pesticide Synergist as Co-Carcinogen

Contractor's Project Director: Mr. B. R. Zeitlin

Project Officer (NCI): Dr. Hans L. Falk

Objectives: To evaluate the carcinogenic, co-carcinogenic, and toxicologic properties of piperonyl butoxide, which has been in common use as a household spray insecticide. Absorption of the pesticide synergist by mammals alters the toxicity of pesticides, but it also may affect the elimination and detoxification of endogenous chemicals, pharmaceuticals, environmental toxicants, including potentially carcinogenic compounds in man's environment.

Major Findings: An analytical method has been in use determing minute amounts of piperonyl butoxide by gas chromatography and electron capture detection. At the higher levels, piperonyl butoxide was found to be somewhat toxic to liver, kidney, and thyroid of the rats, but the presence of DDT made little difference. These experiments on the chronic effects of these chemicals in the diet of rats are more than two years old and have been terminated.

A second set of experiments using different doses of piperonyl butoxide and a carcinogen for the gastric mucosa is under way, and survival of animals in all groups has been excellent.

Significance to NCI Program and Bio-Medical Research: These experiments complement those carried out at Bionetics Research Laboratories, where many other pesticides are under study. The activity of piperonyl butoxide and closely related compounds--all possessing the methylenedioxy phenyl group--has been under study in-house to determine its significance in altering the normal defense mechanisms of the animal to detoxify and eliminate a large variety of environmental or endogenous toxicants.

Hepatocarcinogenicity has been demonstrated in experiments where newborn mice were given injections of Freons and piperonyl butoxide--a combination often encountered in pesticide spray preparations.

With the help of C14-labeled piperonyl butoxide, the fate, distribution, metabolism, and decay of the compound will be studied in greater dimensions than was done until now.

Proposed Course of Project: The experiments will take one more year before completion.

Date Contract Initiated: June 25, 1964

Current Annual Level: January 25, 1968 - January 31, 1969: $87,275

NEW YORK UNIVERSITY (PH43-64-938)

Title: Tumor-Promoting Principles of Tobacco and Tobacco Smoke

Contractor's Project Director: Dr. B. L. Van Duuren

Project Officer (NCI): Dr. Hans L. Falk

Objectives: To isolate and identify chemical components of processed tobacco leaf and smoke which may act as tumor initiators or promoters.

Major Findings: Fractionation of tobacco extract and cigarette smoke condensate has been continued. Work on the tobacco leaf extract has produced a highly active fraction by counter current distribution, which contains thus far unidentified components, but the infrared spectrographic analysis suggests the presence of esters or lactones.

Based on the high incidence of cancer of the oral cavity in betel nut chewers, which probably involves tobacco as the causative agent, tobacco has been treated in a similar fashion as in the preparation of the cud. Tobacco thus treated was found to have carcinogenic properties. These observations have been confirmed by the Contractor, and attempts at isolation of the responsible chemical are also under way.

Of considerable importance are recent findings that certain polycyclic aromatic hydrocarbons found in tobacco smoke and considered entirely inactive by bioassay on mice proved to be effective initiators if croton resin was used as promoter. Compounds tested were benzo(e)pyrene, coronene, anthanthrene, 1,2,3,4-dibenzanthracene, and monomethylanthanthrene. Chrysene, a weak carcinogen, was also included. Of these compounds, 1,2,3,4-dibenzanthracene, monomethylanthanthrene, and chrysene proved to be potent initiators, while benzo(e)pyrene and coronene are borderline cases. Anthanthrene was entirely inactive. These observations make it necessary to take a new look at all the polycyclic hydrocarbons and heterocyclic compounds which have been detected in smoke and to retest them in the presence of promoters. New bioassay systems are under study to determine promoting activity by specific enzyme release from lysozomes. It is still too early to say whether this rapid bioassay technique will be very useful.

A large number of simple and complex phenols have been tested as potential promoters, but except for phenol itself were found to be inactive.

Significance to NCI Program and Bio-Medical Research: The carcinogenic and co-carcinogenic properties of tobacco and tobacco smoke must be identified so that reasonable steps can be taken to alleviate the hazard. It would appear more profitable at this time to concentrate on the identity of the promoters and to ultimately remove them.

Proposed Course of Project: This program should continue for several more years.

Date Contract Initiated: June 24, 1964.

Current Annual Level: December 29, 1967 - December 28, 1968: $104,000

Summary Report
BIOLOGY BRANCH, ETIOLOGY
July 1, 1967 - June 30, 1968

The members of the Biology Branch study fundamental aspects of experimental carcinogenesis. These studies are designed to serve as a basis for the establishment of programs with practical goals. Among these long range goals are the establishment of criteria for distinguishing between carcinogens and non-carcinogens and the development of diagnostic tests and therapeutic procedures for cancer based on host reactions to carcinogenesis.

The Cytogenetics and Cytology Section is responsible for carrying out _in vitro_ and _in vivo_ studies on the mechanisms of chemical carcinogenesis. Carcinogens and related non-carcinogenic compounds are tested by determining whether they induce morphological and biochemical changes in cells; also studied are the effects on these changes of inhibitors and promoters of neoplastic transformation. Among the models used in this work are: cell culture, bacteriophage induction, reversion to prototrophy of bacteria, and newborn, pregnant, and weanling animals.

Study of the early macromolecular events associated with a mitogen used in conjunction with human peripheral blood lymphocytes has continued. Deoxyribonucleic acid synthesis, in mitogen-stimulated human peripheral blood lymphocytes in tissue culture, is dependent upon prior ribonucleic acid and protein synthesis, as determined in experiments with inhibitors of the latter events. The stimulatory effect of two simultaneously added mitogens is not additive, suggesting that part of the population is more than monopotential.

The "reversion" to prototrophy of various types of histidine-requiring mutants of _Salmonella typhimurium_ by known carcinogenic and potentially carcinogenic compounds has been studied in the hope of demonstrating a positive correlation between carcinogenicity for mammals and mutagenicity for _Salmonella_. Such a correlation was found only for certain classes of compounds, comprising by no means all potent carcinogens, and there was poor correlation between carcinogenic and mutagenic activity for weakly carcinogenic compounds. Of the organic peroxides tested only two exhibited mutagenicity for several of the mutant strains used. These are being tested for carcinogenicity in mice.

The induction of temperate bacteriophage by certain classes of carcinogenic compounds has been achieved. The problem of solubilizing non-polar compounds was solved by the use of dimethylsulfoxide. A number of "proximate" carcinogens gave highly significant results, the most active compounds generally possessing N-hydroxy or nitroso function. A number of chemicals and viruses relevant to carcinogenesis have been studied for their teratogenic effect. In addition to using short-term teratogenic experiments, the direct effect of a given compound was studied by applying it to fertilized eggs growing _in vitro_ in a microdrop of tissue culture medium. Additional compounds have been tested, some of which were found to be embryotoxic, while some were teratogenic and others were ineffective. Of the oncogenic and non-oncogenic viruses tested, none were teratogenic. Ethylnitrosourea appears to be one of the most potent teratogens available for over 90 per cent of the live fetuses exhibited malformations. When hamsters were injected on day 8 of gestation, and killed at different intervals thereafter, the types of malformations observed varied: the incidence of malformed but viable fetuses increased with time despite the fact that the most severely malformed fetuses were resorbed. Actinomycin D is also teratogenic and perhaps unique in that unlike other compounds it can be given before maximum differentiation and still produce damage. By use of a variety of injection schedules, LSD was found to be ineffective in terms of toxicity and congenital malformations for the mouse and hamster.

Transplacental carcinogenesis by ethylnitrosourea and related compounds is being intensively studied in mice. Lymphomas, hepatomas, and pulmonary adenomas are readily induced in the offspring of various strains by administration of a single intraperitoneal dose of carcinogen to pregnant females between days 12 and 19 of gestation. Pulmonary tumors are most readily induced on days 16-17, a time when lymphoid proliferation is maximal in the developing thymus, and the marked differences in susceptibility of adult mice of various strains to the induction of these tumors is not observed. Fetuses of all strains tested are equally sensitive at comparable stages of development. On the other hand, hepatomas are observed only in mice of strains which are prone to develop such tumors (e.g., C3H), and male offspring develop a higher incidence of tumors with a shorter latent period than do females.

The study of carcinogen-tissue component interactions during chemical carcinogenesis using cells and tissues cultured in vitro has continued. Progress is being made toward establishing model systems to demonstrate neoplastic transformation, macromolecular effects of carcinogens and non-carcinogens. Transformation is defined as the ability to produce tumors. The previous observations that hamster cells in tissue culture in the presence of known polycyclic hydrocarbons undergo neoplastic transformation has been reconfirmed. Again these cells are free of any indication of viruses that are known to transform hamster cells either in vitro or in vivo. In addition, tissue-cultured cells exposed to other classes of carcinogens, both water-soluble and water-insoluble, appear to demonstrate the same properties of cells which were exposed to the polycyclic hydrocarbon carcinogens. The protective activity of a weakly- or non-carcinogenic polycyclic hydrocarbon in preventing DMBA-initiated cell lysis, inhibition of cell division and DNA synthesis was studied. Induction of benzpyrene hydroxylase activity was studied in cells which were either blocked in the S period with excess thymidine or were subsequently reversed from thymidine block. DMBA inhibition of DNA synthesis and cell lysis were almost completely prevented by simultaneous benzanthracene (BA) treatment. Enzyme activity after the reversal of thymidine blockage reached a maximum after four hours and subsequently declined; addition of fresh inducer produced a reinduction. Furthermore, enzymatic activity was higher during complete suppression than after resumption of DNA synthesis. Thus induction occurs primarily during the GI period of the mitotic cycle, where protection against cytotoxicity and cell lysis is probably initiated.

The *in vivo* animal studies involving potential environmental hazards as well classical carcinogens is proceeding as scheduled. In a study which compared the survival of X-irradiated mice with and without actinomycin D, no change either in the incidence of lymphomas and/or leukemias or the overall survival rates was found. The combination of X-radiation and actinomycin D was much more toxic in male than in female mice. The failure to confirm the skin carcinogenesis effect (prevention of induction of tumors) may be related to the time of the administration of actinomycin D in relation to the time of viremia. Animal studies have been successful in clarifying confusion over structure and carcinogenicity. We have confirmed that both dibenzo [*a*, *l*] pyrene and the compound previously assigned that name in error, dibenzo [*a*, *e*] fluoranthene, are carcinogenic to the subcutaneous tissues of C57Bl/6 mice.

The results of the study of the carcinogenicity of repeatedly heated fats, as in deep fat frying, have been evaluated and prepared for publication. A weak carcinogen appears to be present in fat, both heated and unheated, which is concentrated in the non-urea-adduct fraction. This material or materials is increased in quantity or enhanced in activity by the heating process.

Organic peroxides and related compounds belonging to several different classes of chemical structures have been synthesized by Dr. Daniel Swern, Temple University Contract (PH43-66-68). These chemicals are being administered to mice of several different strains to determine whether these compounds are carcinogenic. The same chemicals are also being tested for teratogenicity in mice and hamsters.

In the Carcinogen Screening Section it was found that non-toxic arginine glutamate prevented the carcinogenic effect of acetamide on the liver of male rats. This property of arginine glutamate appears to be due to its capacity to react with and eliminate ammonium ions. Liver carcinogenesis induced by *N*-2-fluorenylacetamide was inhibited by acetanilide and also by *meta*-acetotoluidide but not by the *para*-isomer. The *ortho*-isomer itself was somewhat toxic and had intermediary inhibiting effect. The corresponding *o*-, *m*-, and *p*- aminobenzoic acids also reduced the toxicity of *N*-2-fluorenylacetamide. The most likely explanation of how these compounds act is that they cause a lower production of the active carcinogenic metabolite *N*-hydroxyfluorenylacetamide, and that they compete with the carcinogen for intracellular and molecular targets. The carcinogenicity of *N*-hydroxy-fluorenylacetamide was also lowered by acetanilide.

Complex kinetics was followed in the complete disappearance of bound carcinogen from DNA, microsomal and supernatant proteins in a 4-week period after a single non-carcinogenic dose of *N*-hydroxy-*N*-2-fluorenylacetamide. However, administration of the tagged carcinogen for 2 weeks yielded higher label and much more prolonged maintenance of isotope, particularly on DNA. A new metabolite of *N*-hydroxy-*N*-2-fluorenylacetamide and of *N*-2-fluorenylacetamide was discovered in serum in the form of the 3-methylthio derivative. In other tests, this compound has been the result of arylation of protein terminal groups, particularly methionine.

Additional controlling elements in carcinogenesis have been successfully attacked. The increasing urinary level of N-hydroxy-fluorenylacetamide known to appear during continuing feeding of this carcinogen or its precursor N-2-fluorenylacetamide is not due primarily to induction of enzymes in liver, as was the common belief, but rather to progressively reduced degradation of the N-hydroxy derivative by bacterial flora in the gut. These important findings were documented in two parallel studies employing germ-free rats on the one hand, and determination of the enzymic complement of gut bacteria capable of reacting with the carcinogenic drug and its metabolites. The participation of bacterial flora in the metabolism and determination of the effective level of drugs opens a novel field in our views of the mechanism of pharmacologic effects. Such considerations are particularly important in view of the frequent utilization of antibiotic drugs affecting bacterial flora in the gut together with other chemotherapeutic agents.

Hypophysectomized rats not susceptible to the hepatocarcinogenicity of fluorenylacetamide showed considerably lower levels of the active N-hydroxy metabolite under sub-acute conditions.

The liver of rats bearing a functional pituitary tumor synthesized twice the amount of albumin as compared to controls, although the rate of albumin synthesis per unit weight of liver or of ribosomal RNA was considerably lower. Liver parenchymal cells were grown successfully in vitro and certain liver carcinogens increased their survival. The cells synthesized serum albumin, a typical function of liver, and possess other enzymes present chiefly in this organ.

Pretreatment of rats with microsomes of liver cancer under several conditions failed to affect the subsequent induction of primary hepatoma with a chemical carcinogen. Chronic treatment of rats with antilymphocytic serum prepared in rabbits had led to poor survival of the rats which showed hepatosplenomegaly. Also, azothioprine (Imuran) given chronically to rats of several strains has resulted in premature death despite careful selection of the dosages administered. In view of the extensive use of antilymphocytic serum and azothioprine in the clinic, additional mechanistic investigations are required. In one test azothioprine has permitted growth of tumor homografts which were rejected without the immunosuppressant.

In the Bioassay Section it has been found that male mice are more susceptible than females to the induction of skin tumors by the two-stage system using 7,12-dimethylbenz(a)anthracene as initiator and croton oil as promotor. Castration of females increases their susceptibility while castration of males results in a slight inhibition. The susceptibility of females fluctuates during the estrus cycle; more tumors are induced when the initiator is applied at the beginning of diestrus than at other times in the cycle.

A technique for cell culture of epidermis has been developed. This technique results in rapid proliferation of epithelial cells with few fibroblastic cells. This technique will permit in vitro studies of carcinogenesis to be done with the same cell type involved in current studies in this laboratory of carcinogenesis in vivo.

A contract with Bionetics Research Laboratories for investigation of chronic toxicity, carcinogenicity and teratogenicity of a large group of pesticides and other environmental agents is approaching completion. Most of the compounds tested appear to be innocuous. Statistical evaluation of the results has just begun.

Activity in the Immunology Section is centered around the development and utilization of immunological and immunochemical tools that may be useful in studying changes in cell antigens due to malignant transformation. One of the major immunological tools that is used in this laboratory in studying antibody-antigen interaction is complement fixation. For a better understanding of the nature and mechanism of the interaction of complement and complement components with antibodies and antigens, the Section conducts a broad program to purify and to characterize components of complement and to elucidate their mechanism of action. The first component of complement has been utilized in the design of a radically new complement fixation test. To understand its action on a molecular basis physical chemical studies into the structure of this molecule are conducted. These studies have been hampered by a lack of adequate supplies of highly purified first component of complement. With the help of the zonal ultracentrifuge, we are designing methods to obtain large quantities of this material in sufficient purity. Some preparations have been obtained that were 80 per cent pure. We have also been able to show with these preparations that every C'1 molecule in these preparations is capable of interaction with antibody-antigen complexes and are capable of leading to cell death. This finding indicated that under some conditions the first component of complement is 100 per cent efficient in the cytolytic system. Studies on the site of synthesis of this component have shown that human C'1 is synthesized in the intestine. It was found that in humans the colon is a major site of C'1 production. These _in vitro_ studies on the synthesis of the first component of human complement were performed by the use of radioactive amino-acids and by the use of biochemical inhibitors of synthesis such as puromycin and actinomycin D. In these studies we have obtained information that indicates that puromycin may under some conditions enhance the synthesis of a protein.

We have continued to apply the C'1a fixation and transfer test to the analysis of cell surface antibody and antigen reactions. This highly sensitive and precise tool is now being applied to studies of antigens and antibodies induced by murine leukemia viruses. The C'1a fixation and transfer test is a convenient method to detect and distinguish antigens induced by the different leukemia viruses. Quantitative measurements of rabbit antibody of the IgM class to Rauscher leukemia cells by C'1a fixation and transfer and immune cytolysis indicated that approximately 3,000 complement-fixing sites were generated on the cell surface for each cytolytic site. Efforts are also being made to study the relationship between virus antigen and the antigen induced on the cell surface by these viruses. For this purpose studies were initiated to find efficient ways of coupling soluble antigens to cell surfaces. Preliminary findings indicate that carbodiimides may serve as useful coupling agents.

A contract (PH43-66-935) has been initiated with the Cordis Corporation to purify complement components and to standardize the reagents used in their assay. The contractor has supplied two shipments of all nine components of human complement and one shipment of the first and second components of guinea pig complement. Most of these components have been satisfactory.

In the Office of the Chief the search for tumor specific immunity has shifted from studies of humoral antibodies to studies of cellular immunity. Malignant hepatomas induced in strain 2 guinea pigs have been transplanted in this strain and have been shown to possess antigenic activity in the syngeneic host. Antigenicity in one transplantable line has been demonstrated both by delayed cutaneous hypersensitivity and protection tests. Intradermal injection of living tumor cells is an effective technique of immunization and has the advantage that the resulting tumor papule usually regresses spontaneously. Initiation of intradermal immunization four days after intramuscular challenge with live tumor cells produces a measureable state of immunity. The results of these studies should provide an experimental basis for the demonstration of tumor specific antigens in man as well as a basis for clinical trials of immunotherapy. Experiments designed to test the applicability of information obtained from the guinea pig studies to human cancer have been discussed with several members of the Clinical Staff at the National Cancer Institute. Conditions for separating adenovirus T antigens from Virion antigens have been established. Four hundred sera of cancer patients with age-matched controls were tested for antibodies to purified adenovirus T antigen. No antibodies to adenovirus T antigen were found in the sera of these patients.

A major effort of the Molecular Separations Unit's program is devoted to the application of its special instrumentation to biochemical and biophysical studies associated with carcinogenesis. The zonal ultracentrifuges are unique in this respect, and most of the program development is related to this equipment. One new rotor, a high-speed titanium version of the B-XIV, has been put into routine service and has considerably expanded capabilities for working with various proteins. Most other new developments have been associated with the use of new gradient preparations, both in form and composition. The new approaches have offered facility in the study of such biological materials as isolated chromosomes, mammary tumor virus, and isolated mitochondria. Special attention has been given to the use of gradients that are isotonic with regard to mammalian tissues, a condition that is of particular importance in studies on mitochondria and certain virus preparations. Considerable progress has been made in developing new methods for extracting and studying very small amounts of DNA. Recoveries of DNA have been increased to about 80 to 90 per cent efficiency and we can reliably work with amounts as small as about five micrograms of DNA in a sample. This has had considerable impact on investigations pertaining to mitochondrial DNA and the biological significance of satellite DNA.

The Ultrastructural Unit, Office of the Chief, Biology Branch, has been concerned with three projects. 1) The action of antibody and complement on cell membranes, as a method of studying antigens of cell surfaces and also the structure of membranes in general. 2) The response of the intact animal to chronic administration of various doses of the hepatotoxin and hepatocarcinogen, aflatoxin B-1, with characterization of the morphological changes of the liver. Aflatoxin binds to DNA and inhibits DNA dependent RNA synthesis in a manner similar to actinomycin D. Nuclear changes reflect this similarity. Induction of smooth membrane proliferation, characteristic of detoxifying mechanism seen with many toxins, was marked. The mitochondria showed changes which have been described in cortisone administration, which, in light of the adrenal hypertrophy, suggests further studies on the hormonal influence on liver carcinogenesis are in order. 3) Studies on mitochondrial DNA synthesis and replication in rapidly proliferating liver cells have been started, using both fractionation and autoradiographic techniques.

Contract activity consists of three contracts, two of which are aimed at developing *in vitro* assay methods, concentrating on mouse mammary gland tissue and lung organ cultures. Known carcinogens and non-carcinogens are used as test agents. The third contract is concerned with the establishment of early morphological and biochemical characteristics of a preneoplastic lesion in the liver. Chemicals tested or being tested are aflatoxin B-1, griseofulvin, thiuram and thioacetamide.

Serial No. NCI-4634
1. Biology Branch, OASDC, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Immunochemical Studies of Normal and Neoplastic Tissues

Previous Serial Number: Same

Principal Investigator: Herbert J. Rapp, Sc.D.

Other Investigators: Berton Zbar, M.D., Winthrop H. Churchill, Jr., M.D., Barry S. Kronman, M.D., and Tibor Borsos, Sc.D.

Cooperating Units: For supplies of starting material used in purification of T antigen: Flow Laboratories, Dr. R. Gilden and the National Institute of Allergy and Infectious Diseases, Dr. R. Huebner.

Man Years

Total:	5.0
Professional:	4.0
Other:	1.0

Project Description

Objectives:

The primary objective of this project is to establish conditions and methods suited to the detection of tumor-specific antigens and antibodies. A long range objective is to develop diagnostic tests for cancer based on the assumption that tumors contain unique antigens capable of eliciting the formation of tumor-specific humoral antibodies or of causing the appearance of cells which react specifically with tumor antigens. Transplantable syngeneic guinea pig tumors will be used to investigate conditions for effective immunotherapy of solid tumors.

Methods Employed:

Malignant hepatomas have been induced in strain 2 guinea pigs by feeding them diethylnitrosamine (DEN) in their drinking water. Three of these tumors are being maintained in transplant and ascites variants by periodic transplantation of the tumors and, when available, of their ascites variants. The antigenicity of one of these tumors (line 1) (currently in the 21st transplant generation) has been studied by transplantation techniques and delayed cutaneous hypersensitivity. Sera from guinea pigs immunized with this tumor have been tested for antibody to the tumor by C'la fixation and transfer (See project NCI-4637). The influence of immunization by intradermal injection of tumor cells on the growth of an intramuscular transplant of the same tumor is also being explored.

This tumor is being maintained in short and long term tissue culture. More hepatomas are being induced by feeding strain 2 guinea pigs diethylnitrosamine.

Antisera to the guinea pig hepatomas have been prepared by immunization of rabbits with homogenates of the tumor tissue. Hepatomas have been produced in rabbits by feeding them DEN. Antisera to the rabbit hepatomas have been prepared by immunization of rabbits with rabbit tumors.

Sephadex chromatography has been used to separate T and V antigens of adenoviruses. Sera from patients with malignancy have been tested by a microcomplement fixation test for the presence of antibodies to purified adenovirus type 7 T antigen.

Major Findings:

1. One (line 1) of the three available transplantable hepatomas has been shown to have tumor antigens as judged by the criterion of protection. Studies of the other two are just being started.

2. This tumor is an adenocarcinoma of the liver. Its morphology has remained stable. The ascites variant of this tumor provides large numbers of single tumor cells.

3. Strain 2 guinea pigs, immunized with tumor administered either intramuscularly or intradermally develop a specific skin reaction to antigens of this tumor.

4. The rate of development of this skin reaction, its histologic features and specificity are characteristic of delayed hypersensitivity.

5. Animals with a positive skin reaction are more resistant to growth of this tumor than are control animals.

6. Immunization of strain 2 guinea pigs with normal strain 2 tissues or another strain 2 tumor (induced by methylcholanthrene) does not influence the size of this skin reaction or the growth of a subsequent inoculum of tumor cells.

7. Intradermal injection of living tumor cells is an effective technique of immunization and has the advantage that the resulting tumor papule usually regresses spontaneously and does not need to be excised. Injection of the same number of cells intramuscularly will cause a tumor which grows progressively and eventually kills the animal.

8. Guinea pigs immunized by either intramuscular or intradermal injection of tumor are protected against a subsequent inoculum of the tumor whether it is given intradermally, intramuscularly or intraperitoneally.

9. Intradermal immunization is an effective form of immunotherapy. Animals were given tumor on day 0 and intradermal immunization was begun on day 4. Results depended on the size of the tumor inoculum given initially. Immunization did not influence the growth of tumors in animals which received an initial inoculum of 3×10^6 tumor cells. Growth of tumors in animals initially injected with 3×10^5 tumor cells was slower in the immunized group than in the control group. Tumors in two of six immunized animals eventually regressed. In animals receiving an initial inoculum of 3×10^4 cells, tumor appearance was delayed and the rate of growth retarded by immunization. Immunized animals lived longer in all groups except the one which received an initial inoculum of 3×10^6 tumor cells.

10. Feeding guinea pigs diethylnitrosamine, does not affect their ability to develop positive skin reactions to this tumor and increased resistance to the same tumor.

11. The ascites variant has been maintained as an epithelial cell line in tissue culture for six months. Reinjection of these tissue culture cells produces a tumor of similar morphology to the parent tumor.

12. As measured by delayed hypersensitivity there is significant destruction of the activity of the tumor antigens by a variety of relatively mild treatments.

13. Attempts to transform guinea pig lymphocytes by exposing them to syngeneic tumor cells have been unsuccessful.

14. Conditions for separating T from V antigen have been established. Four hundred sera of cancer patients with age matched controls were tested for antibodies to adenovirus type 7 T antigen. No antibodies to adenovirus T antigen were found in the sera of these patients.

15. Tumor specific antibody to guinea pig hepatoma could not be detected in rabbit antisera by quantitative complement fixation.

Significance to Bio-medical Research and the Program of the Institute:

The availability of a transplantable guinea pig tumor permits experimentation which is difficult to perform in a mouse tumor system. Since studies in cellular immunity have been performed to the greatest extent in the guinea pig, this tumor system will facilitate studies in tumor immunity. This tumor system will permit extension of studies of tumor cell interaction *in vitro* to the guinea pig. Previously studies have been confined to mouse and rat tumors.

The demonstration that tumor antigens can be detected by delayed hypersensitivity is of major importance. This model system provides an experimental basis for similar studies in man. This skin testing technique can be used to study cross reactions among different tumors and as an assay for antigen activity during purification of tumor antigens.

The intradermal protection tests permit rapid comparison of different immunizing techniques and should be useful in establishing optimal conditions for immunization against tumor.

This guinea pig tumor system is suitable for studying immunotherapy of established tumors. The results of such studies should provide an experimental basis for clinical trials.

In addition to our tests for antibody in sera of cancer patients to adenovirus type 7 T antigen, tests with other types of adenovirus T antigens (types 2 and 12 as well as 7) have been carried out in other laboratories using aliquots of the identical sera used here. Moreover, tests were performed with purified T antigens obtained in a variety of ways. In these other studies immunofluorescence as well as complement fixation has been used. Since the results of all of these tests have been negative, it can be concluded that the sera of humans with cancer do not contain detectable antibodies to adenovirus T antigens. There is no evidence, therefore, that adenoviruses oncogenic in the hamster are responsible for causing any human tumor.

Proposed Course:

Further studies on other diethylnitrosamine induced tumors will be initiated as these tumors become available. The purpose of these investigations will be to determine the immunological relationship among these different tumors. The techniques worked out for transplantable tumors will be applied to newly induced tumors before transplantation. This tumor (line 1) will be used to study conditions required for effective immunotherapy of established solid tumors.

Attempts will be made to develop *in vitro* methods to detect tumor antigen. Studies to establish conditions for purification of tumor antigens are under way. The possibility of using tissue culture cells as a starting material for fractionation studies will be investigated. The potency of purified fractions, as they become available will be tested by protection and skin reaction techniques.

The search for heterologous tumor specific antibodies is being discontinued.

Publications

Zbar, B., Rapp, H. J., Huebner, R. J., and Borsos, T.: Adenovirus type-7 T antigen: Separation from hexon antigen by gel filtration. A preliminary report. *J. Nat. Cancer Inst.* 40: 643-646, March 1968.

Serial No. NCI-4635
1. Biology Branch, OASDC, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Biologic Activities of Complement

Previous Serial Number: Same

Principal Investigator: Herbert J. Rapp, Sc.D.

Other Investigators: Winthrop H. Churchill, Jr., M.D., and Tibor Borsos, Sc.D.

Cooperating Units: Dr. Ronald T. Rolley, Surgery Branch, NCI

Man Years

Total:	1.5
Professional:	1.0
Other:	0.5

Project Description

Objectives:

To characterize the sequence of action of mouse complement components and to identify wherein it differs (if at all) from the sequence of action of guinea pig complement components. To identify the complement components influenced by hormones; to identify other factors hormonal or otherwise which influence the activity of mouse complement components; to determine the effect of complement deficiency on biologic function.

Methods Employed:

Techniques developed for guinea pig C' component measurements have been adapted for measurement of mouse complement. The techniques include component analysis on a molecular basis and quantitative analysis of kinetics of interaction of C' components with antigen-antibody complexes. Adult mice have been thymectomized to study the effect of ablation of this organ on the activity of mouse complement. Mice were thymectomized by Dr. Ronald T. Rolley, a member of the Surgery Branch, NCI, who is collaborating with us on this project.

Major Findings:

1. We were unable to confirm our preliminary observations which showed that thymectomy influenced mouse complement. The initial observations were made on a group of mice with chronic pneumonia. These observations when repeated in healthy mice, could not be confirmed. Other experiments, showed that chronic pneumonia is associated with depressed activity of mouse complement.

2. We attempted to study the influence of hypophysectomy on mouse complement because of our finding that sex hormones influence mouse complement activity. These experiments were abandoned because of the difficulty of obtaining and maintaining hypophysectomized mice.

3. The formation of a thermostable cell intermediate by serum from mice genetically deficient in C'5 has been confirmed. This intermediate is destroyed by 0.01M EDTA and is not analogous to either of the thermostable intermediates which are generated by guinea pig complement. Addition of C'1a to the thermostable intermediate, after treatment with 0.01M EDTA, does not restore the activity of this intermediate.

4. The class of antibody used to make EAC'1a42a cells influences C'EDTA titer of mouse complement.

Significance to Bio-medical Research and the Program of the Institute:

The availability of mice deficient in one or more components of complement permits design of experiments in which *in vivo* complement antagonists are not required to achieve complement depletion. These mice have been used to investigate the biologic functions of complement by a number of investigators. The following biologic functions have been studied: graft rejection, rejection of allogeneic and syngeneic tumors, Arthus reactivity, clearance by the reticuloendothelial system, resistance to infection, Masugi nephritis, and induction of amyloid. With the exception of resistance to allogeneic tumors, none of these parameters have been influenced by the presence or absence of C'5 in the mouse serum. It may be that complement components which precede C'5 are of importance in some of these biologic functions. This possibility, however, cannot be studied until other methods of inactivating complement components are developed.

The possible influence of hypophyseal hormones on mouse complement needs further study because the previously observed effects of sex hormones on mouse complement could either be direct or mediated through hypophyseal hormones.

Study of the thermostable intermediate generated by mouse serum may provide insight into the molecular mechanisms of mouse complement.

Proposed Course:

Further work on the project has been suspended. It may be resumed in the future if sufficient personnel are available. At that time we would continue to investigate the influence of hypophyseal hormones on mouse complement, the thermostable intermediate of mouse complement and the influence of antibody on the C'^m EDTA titer.

Serial No. NCI-4640
1. Biology Branch, OASDC, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Fractionation and Studies on Mouse Liver Mitochondria

Previous Serial Number: Same

Principal Investigators: Howard E. Bond, D.V.M., Ph.D., John A. Cooper, II, Ph.D., and Doris P. Courington, M.D.

Other Investigators: None

Cooperating Units: Ultrastructural Unit, Biology Branch, ET, NCI

Man Years:

Total:	1.2
Professional:	1.0
Other:	0.2

Project Description

Objectives:

The objectives of this project are to isolate mitochondria free of contamination and to fractionate them on the basis of size for further study. Investigations into the metabolic and functional state of the mitochondria are the basis for studies on their biogenesis and their involvement in the neoplastic condition.

Methods Employed:

The main methods used are the zonal centrifugation techniques. Both the low-speed A-IX rotor and the high-speed B-rotors are employed depending upon whether or not a rate-zonal or isopycnic isolation is desired. Radioactive precursors are incorporated into the mitochondria in vivo in many experiments. The isolated mitochondria and their constituents are subjected to further study using a variety of conventional laboratory procedures, radioactivity counting methods, electron microscopy, and specialized centrifugation methods developed in the laboratory for investigations on DNA (fixed-angle rotor density gradient centrifugation).

Major Findings:

A major emphasis has continued to be placed upon studying the relationship of the mitochondrial DNA to the size of the mitochondrion. Preliminary data indicate that the ratio of DNA to protein (hence, size) remains relatively constant in all of the size classes. Further, the specific activity of the DNA after precursor incorporation also appears to remain constant suggesting that there is no preferential DNA synthesis in any particular size class. This latter point is being checked with greater precision using autoradiographic techniques in conjunction with the electron microscope. DNA with a very high specific activity which is distinctly not nuclear has been observed during these studies. It would appear that this material is associated with membranes in the microsomes, and the possible relationship of it to mitochondrial DNA is being investigated.

The isolation techniques used most of this project year have involved sucrose gradients that are hypertonic to the mitochondrion. Mitochondria are known to be susceptible to osmotic effects in sucrose, and they obviously must be affected to some extent by the isolation procedures. The problem could be ignored largely in the studies on their DNA, but it must be taken into account for any meaningful study to be undertaken on the functional state of the isolated mitochondria. Presently, new methods of isolation are being investigated which avoid the use of hypertonic gradients and should provide mitochondria in a more nearly native functional state.

Significance to Bio-medical Research and the Program of the Institute:

The biogenesis of mitochondria is still a largely unsettled question. Although it is generally thought that their formation is from fission of pre-existing mitochondria, this fails to explain certain extranuclear genetic phenomena as well as their apparent *de novo* formation under specialized experimental conditions. Mitochondria have been seen to divide by fission, so this is known to occur in the cell. However, the presence of an alternative method of biogenesis, if it can be shown to occur, would be highly significant. It is felt that the mitochondrial DNA will be a useful marker to follow in the study of this problem.

A number of metabolic derangements involving mitochondria have been described in various forms of cancer. The ability to isolate mitochondria on the basis of size will provide a useful refinement in studying the functional state of these structures in such neoplasms.

Proposed Course:

The project will be continued on a long-term basis renewable yearly.

Serial No. NCI-4641
1. Biology Branch, OASDC,
Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Cellular Protein Fractionation Studies

Previous Serial Number: Same

Principal Investigator: Howard E. Bond, D.V.M., Ph.D.

Other Investigator: John A. Cooper, II, Ph.D.

Cooperating Units: Laboratory of Biology, GLC, NCI

Man Years:	
Total:	0.4
Professional:	0.4
Other	0.0

Project Description

Objectives:

The objectives of this project are to isolate cellular proteins in a reproducible manner and to establish identifying characteristics for them so that they can be recognized subsequently in complex mixtures. This will allow further study such as on the kinetics of synthesis and turnover without the necessity of extensive isolation procedures. The approach is being used to recognize cellular protein changes in mouse hepatomas in an attempt to assess the biological significance of the changes.

Methods Employed:

Liver tissue from the mouse is being used in the present study. The soluble supernatant is prepared from homogenates and chromatographed on cellulose ion exchange material using a special gradient machine and complex gradients. Fractions obtained from the column are further investigated by acrylamide gel electrophoresis and immunochemical procedures such as gel immunodiffusion and immunoelectrophoresis. Other typical laboratory procedures are used as appropriate.

Major Findings:

Through the use of chromatograph techniques employing complex gradients, methods have been developed whereby the soluble cellular proteins of the liver can be chromatographed with a high degree of resolution and reproducibility. The result has been to produce a "profile" in the normal tissue against which experimentally altered or pathological changes can be distinguished. Application of these methods are being used to study the C_3H-A^{vy} mouse supplied by Dr. Heston from the Laboratory of Biology, GLC, NCI. Distinct protein alterations have been detected in tumor nodules taken from this mouse, and refinements of the chromatographic procedures have been made to isolate the proteins showing change. In addition, the first series of antisera have been made against the crude fractions. These will be used to help specify the proteins in further purification for the development of more specific immunochemical reagents.

Significance to Bio-medical Research and the Program of the Institute:

Many varied and complex protein changes are either known or assumed to occur in the cancer cell. Some enzyme changes have been described on the basis of their detectable activity. However, the vast number of cellular proteins have no known activity ascribed to them, and these could undergo dramatic change relatively undetected. The methods used here, by a combination of isolation and characterization, show promise of filling some of the gap in knowledge by identifying proteins undergoing change and by synthesizing an identifying label for them immunochemically. Once a means of tagging the proteins of interest is available, complicated experiments involving kinetic changes should become comparatively easy.

Proposed Course:

This project is being curtailed during this year. The nature of the study is such that it is very time consuming, and the resources of the Unit from the standpoint of time and personnel are insufficient to meet the requirement. The methods have been developed and the immune sera are stored frozen. The project will be reactivated as soon as possible.

Serial No. NCI-4642
1. Biology Branch, OASDC,
   Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Study of the Ultrastructural Changes Induced in the Calf Liver by Aflatoxin

Previous Serial Number: Same

Principal Investigator: Doris P. Courington, M.D.

Other Investigators: Paul Lynch, D.V.M., USDA

Cooperating Units: Department of Dairy Research, USDA, Beltsville, Maryland

Man Years:
Total: 0.75
Professional: 0.25
Other: 0.50

Project Description

Objectives:

To study the morphological changes associated with chronic administration of aflatoxin at different dose levels in the intact animal and to correlate these with biochemical profiles of each animal; to study the fate of aflatoxin in this species and to compare the response to other species.

Methods Employed:

Aflatoxin is fed in varying concentrations in the grain and/or forage or by bolus and various liver function tests are performed at regular intervals until sacrifice. The intramural portion utilizes standard techniques of electron microscopy, including quantitative evaluation of cytoplasmic structures by recently described techniques.

Major Findings:

The earliest changes in aflatoxin are first found in the peripheral portion of the lobule. With chronic administration, changes are seen throughout the lobule. The severest changes seen in a given animal roughly correlate with the total dose administered. Changes do not equally involve all cells at a given time. The nuclear changes are comparable to those described with actinomycin and the molecular mode of action is considered to be comparable to that of actinomycin. The cytoplasm showed marked proliferation of the smooth membranes at the expense of rough endoplasmic reticulum which was generally decreased and exhibited marked cisternal dilatation. Previously described alteration in size and number of mitochondria resemble changes seen with cortisone administration. This finding is especially interesting in view of the fact that all animals investigated to date show marked hypertrophy of the adrenal cortex. In contrast to the early phase where acute necrosis is pronounced, necrosis was not prominent and cytoplasmic lysosomes were decreased.

Significance to Bio-medical Research and the Program of the Institute:

Although molecular mechanisms are more difficult to evaluate in chronic studies, the importance of these cannot be overstressed when one is dealing with a hepatotoxin such as aflatoxin B-1 where long term, low dose may be important in human exposure. The marked adrenal hypertrophy and its role in liver metabolism can only be evaluated in such studies.

Proposed Course:

The morphological aspects of the project is completed and a manuscript is in preparation.

Serial No. NCI-4653
1. Biology Branch, OASDC, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Fractionation and Characterization of Cellular DNA

Previous Serial Number: NCI-4714

Principal Investigators: Howard E. Bond, D.V.M., Ph.D., and John A. Cooper, II, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1.4
Professional: 0.3
Other: 0.2

Project Description

Objectives:

The objectives of this project are to fractionate as completely as possible the DNA components of the mammalian cell so that the involvement of each of the substituents in the biology of the cell can be more effectively studied. Essentially, the attempt is being made to fractionate the mammalian genome to isolate genetic markers suitable for biochemical study. Also, an objective of the study is to determine the significance of redundant DNA such as mouse satellite DNA and its relationship to the remainder of the genome.

Methods Employed:

Purification of DNA-containing subcellular constituents was performed by zonal centrifugational techniques, and the DNA was extracted and isolated by somewhat complicated procedures developed in this laboratory. Subsequent studies were conducted by conventional laboratory procedures with heavy reliance placed upon analytical ultracentrifugation.

Major Findings:

Although interest is not confined exclusively to the mouse satellite DNA, the major concern of this project is still centered about this unusual material. At present, there is no knowledge of any genetic activity associated with the satellite DNA, and there is no explanation for its redundancy. A search for functional activity associated with the material is continuing. In a previous report, evidence for the random distribution of the satellite throughout the genome was given. An attempt to prove the assertion is being undertaken by studying the satellite distribution in isolated chromosomes. Very preliminary data at the present time suggests that the satellite is randomly distributed as claimed.

A major effort over the past year has been devoted to completely revising and improving methods for extracting DNA from mammalian tissues. It was found that the commonly known methods gave such poor recoveries, that the extraction procedure was the factor limiting many experiments that might otherwise be done. We appear to have raised the extraction efficiency to about 80 to 90 per cent recovery and have devised methods of working effectively with amounts of DNA as low as about five micrograms total in a sample. This has provided means for studying subfractions of materials where previously the entire organ was barely sufficient.

Significance to Bio-medical Research and the Program of the Institute:

The mammalian genome is very complex, and it must consist of a large heterogeneous population of DNA molecules specifying distinct genetic activities. Efforts are being made here to find ways to subfractionate the genome to reduce the complexity and make it possible to do meaningful biochemical genetic experiments in the mammalian system. Further, subfractionation should provide a means for investigating specific interactions of materials like chemical carcinogens with specific components within the complex genome

Proposed Course:

To be renewed yearly.

Publications

Bond, H. E., Flamm, W. G., Burr, H. E., and Bond, S. B.: Mouse satellite DNA. Further studies on its biological and physical characteristics and its intracellular localization. J. Mol. Biol 27: 289-302, 1967.

Serial No. NCI-4654
1. Biology Branch, OASDC, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: DNA Synthesis and Replication of Mitochondria

Previous Serial Number: None

Principal Investigator: Doris P. Courington, M.D.

Other Investigators: Howard E. Bond, D.V.M., Ph.D., and John A. Cooper, II, Ph.D.

Cooperating Units: Molecular Separations Unit, Biology Branch, NCI

Man Years:

Total:	0.75
Professional:	0.25
Other:	0.50

Project Description

Objectives:

To study the details of DNA synthesis and structure in mitochondria and the replication of mitochondria in mammalian cells; to study the effect of various carcinogens on these characteristics of mitochondria.

Methods Employed:

Ultracentrifugation techniques are used to separate mitochondria from other subcellular fractions and to further characterize them by banding. Tritiated thymidine is used to study DNA in both subcellular fractions and in rapidly proliferating liver cells obtained by partial hepatectomies.

Major Findings:

Distribution of the tritiated thymidine label in fractionation studies and in autoradiography substantiate the concept that mitochondria can replicate by division. The appearance of labeling in other subcellular fractions suggests other mechanisms in certain conditions. Findings are discussed in more detail in the report from the ultracentrifugation unit.

Significance to Bio-medical Research and the Program of the Institute:

Findings in recent years have indicated intramitochondrial DNA is probably a universal characteristic. A circular configuration, previously found only in viruses and bacteria, has been demonstrated in mitochondria from mouse and rat liver and from certain tissue culture cells. Renaturing characteristics of the heat or alkali-denatured DNA from mitochondria further distinguish it from nuclear DNA. The possible role of cytoplasmic genetic mechanisms is obvious but has only been established with certainty in studies with neurospora. Although alterations in mitochondrial enzymes have been reported in certain carcinogens, the possibility that ultimate changes may be mediated through mitochondrial DNA has not been evaluated and seem important in view of the known differences in mitochondria and mitochondrial functions in tumors and normal tissues.

Proposed Course:

This project will be continued on a long-term basis renewable yearly.

Serial No. NCI-4655
1. Biology Branch, OASDC,
   Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Ultrastructural Studies on the Action of Complement and Antibody on Cell Membranes in Immune Hemolysis

Previous Serial Number: None

Principal Investigator: Doris P. Courington, M.D.

Other Investigators: Carl G. Evans, M.S., Michael M. Frank, M.D., Tibor Borsos, Sc.D., and Herbert J. Rapp, Sc.D.

Cooperating Units: Immunology Section, Biology Branch, NCI

Man Years:

Total:	1.5
Professional:	0.5
Other:	1.0

Project Description

Objectives:

To further investigate the morphological manifestations of the action of complement and antibodies on cell membranes in direct and indirect hemolysis; to use the method to gain further information regarding the structure of cell membranes of different types, including bacterial membranes.

Methods Employed:

Complement components and antibody of desired titers from various sources are reacted with appropriately washed cells to produce immune hemolysis. Osmotically lysed cells are used as controls. Fragments of cell membranes are stained negatively with 2 per cent phosphotungstic acid and are examined with the electron microscope. "Lesions" are quantitatively evaluated from the negative by examination under a dissecting microscope and membrane area is calculated by planimeter measurements. Qualitative study of the membrane and lesion is made on electron micrographs at appropriate magnifications.

Major Findings:

Previous reports indicate that the action of complement (C') and antibody on red blood cells or bacteria results in formation of discrete holes in the cell membranes. We have observed structures indistinguishable from holes located on the supporting substrate as well as on the membranes. This finding has been tentatively interpreted as representing a conformation change which occurs with the reaction of the complement and antibody, producing a structure with distinct characteristics which are enhanced by the negative staining technique. Examination of unstained preparations tend to support this concept, as does also the reported finding by others that the late-acting components of complement are inhibited by iron chelating compounds (inferring a structure with an innate electron density).

Significance to Bio-medical Research and the Program of the Institute:

This approach offers molecular information about the antigen-antibody reactions on cell surfaces and about complement fixation. It offers some indirect information regarding the structure of membranes. The previously accepted "universal" unit membrane (tri-laminae membrane) is currently under re-evaluation. The lipid content of membranes from different sources varies widely. Recent studies by Singer and Leonard indicate that 60 per cent of the lipids can be removed from red cells by phospholipase A, with an "intact" membrane remaining when examined by the phase microscope. This infers that the polar groups of the lipids previously felt to be covered by protein and carbohydrate layers are in fact exposed. Other optical techniques (circular dichroism measurements) indicate that fixatives routinely used in electron microscopy markedly alter properties of cell membranes. Hence systems using unfixed membranes and defined enzyme systems are important for further study of membrane structure.

Proposed Course:

The development of replica techniques, especially the freeze-etch technique, which allow three dimensional studies will be necessary to help resolve some of the problems. Localization of antibodies by tagging (radioactive and electron-dense) and the use of ultrastructural cytochemical techniques for localization of enzyme activity are anticipated.

Serial No. NCI-4713
1. Biology Branch, CACDC,
Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The NIH Zonal Ultracentrifuge Project

Previous Serial Number: Same

Principal Investigator: Howard E. Bond, D.V.M., Ph.D.

Other Investigators: John A. Cooper, II, Ph.D., Doris P. Courington, M.D., Charles W. Boone, M.D., Ph.D., Harvey R. Colten, M.D., Norman P. Salzman, Ph.D., William T. Hall, Ph.D.

Cooperating Units: Ultrastructural Unit, Biology Branch, ET, NCI; Cell Biology Section, Viral Carcinogenesis Branch, ET, NCI; Immunology Section, Biology Branch, ET, NCI; Laboratory of Biology of Viruses, NIAID; Laboratory of Biology, GLC, NCI.

Man Years:
Total: 2.0
Professional: 1.0
Other: 1.0

Project Description

Objectives:

The objectives of the NIH Zonal Ultracentrifuge Project have remained the same as in previous years. The main purpose of the project is one of developing methodology in which the zonal centrifuges are uniquely suited for the study of chemical and biophysical events associated with cancer. Since zonal centrifuges are still relatively new and few in number, this has led to the establishment of several cooperative studies with other investigators both within the National Cancer Institute and within other institutes. Initially these investigations have been in the nature of feasibility studies to test the applicability of the centrifugal methods to particular problems. Individual projects have evolved from these investigations once the utility of the approach was shown, and these are reported separately. Accordingly, the NIH Zonal Ultracentrifuge Project continues to service other projects by developing methods for their effective study. It is the nature of the developmental work that is reported here.

Methods Employed:

The main methods employed are those relating to the use of the zonal centrifuge equipment. Representatives of nearly all of the currently available zonal rotors are available in the laboratory, and they are used in applications where each is best suited. A relatively new development is the extensive use of the titanium version of the B-XIV rotor. This is seeing service in certain high-speed applications involving the separation of macromolecules. Conventional ultracentrifuge and other routine laboratory procedures continue to augment studies done with the zonal centrifuges.

Major Findings:

A major concern over the year has been the continuing search for new gradient materials and methods of using them to preserve morphology and biological activity of separated particles. The problem has been partially solved in the case of whole cells by using Ficoll gradients made up in spinner tissue culture medium. Functionally intact cell populations have been separated on such gradients. However, the high viscosity of Ficoll solutions has introduced problems which make these gradients less than perfect, and the search for other materials is continuing.

Special gradient problems also have evolved in studies on the mouse mammary tumor virus, investigations that are being conducted in association with Dr. Hall in the Laboratory of Biology, NCI. The virus is being separated and purified by a rate zonal centrifugation followed by isopycnic banding. However, the virus is comparatively intolerant of an environment having a high osmolarity such as found in usual banding media. This problem can be overcome by using performed Ficoll gradients, but the very high viscosity of Ficoll solutions at the banding density makes it relatively unsuitable for this purpose. An answer to the problem has been found by using heavy water as the solvent for the Ficoll and thereby reducing the amount of Ficoll needed for banding to about one half that normally required. The mouse mammary tumor virus is presently being obtained in highly purified form from the banding step where it is found in a narrow dense band. Bioassay studies are currently underway, and when these are completed, the investigations will be evolved into a separate project pertaining to the biology of the mammary tumor virus in the host-virus relationship.

Problems pertaining to gradient composition have been a central issue in other studies undertaken during the year. One of these has been concerned with the separation and isolation of mammalian cell chromosomes, an accomplishment which should be of inestimable importance for research on the mammalian genome. A chief difficulty has been that of retaining the chromosome morphology and preventing aggregation. Research has been mounted in cooperation with Dr. Norman Salzman in the Laboratory of Biology of Viruses, NIAID to overcome the problems using special buffer conditions that he has developed. Although some aggregation still exists and further development is needed, the initial separations appear highly successful particularly among the smaller chromosome classes. The low-speed zonal rotor has been used for these preliminary studies, and it appears to offer great promise for the continuation of the work.

Interactions between the particle and the gradient also has become an important source of inquiry regarding mitochondrial separations. Extensive work has been done on isolating mouse liver mitochondria on the basis of size, a project that is being reported separately. Unfortunately, the mitochondrion acts as an osmometer and undergoes change in the hypertonic sucrose gradients used. In the nature of the work to the present time, this problem could be largely ignored. However, as the nature of the studies are changing, the need to avoid the hypertonic changes are becoming more important. Consequently, investigations are underway with different gradient materials which will allow rate zonal and isopycnic banding centrifugation in effectively isotonic media.

Considerable study has been spent on developing special gradient conditions for separating proteins in the zonal rotors. This has been not so much from the standpoint of the gradient solute composition but more from that of the gradient shape and position in the rotor. Using the high-speed titanium version of the B-XIV rotor, it has been possible to obtain partial resolution of materials having a sedimentation coefficient as low as 5 S. This appears to be the limit of our capabilities at the present time, although newer equipment becoming commercially available could possibly extend the range. With the existing facilities it has been possible to work quite effectively with 7 and 19 S molecules, and an application of this has been made in cooperation with the Immunology Section in studying the first component of complement (C'1). The details of the investigations will be reported by that laboratory.

Significance to Bio-medical Research and the Program of the Institute:

The use of centrifugal methods of separation has assumed great importance in most of the currently popular fields of biological research. This is particularly true in the area of molecular biology, and it is rare to find a study here that does not take advantage of the methods during some aspect of the research. The zonal centrifuges, primarily because of their greater capacity and resolution over conventional techniques, have extended the capabilities of centrifugal methods to a point that they frequently are a decisive factor in a successful experiment. The continuing development of methods and the application of zonal techniques to problems of biology is sustained by the importance of the problems that can be solved.

Proposed Course:

The project will continue renewable yearly with no basic change in objectives.

Publications

Boone, C. W., Harell, G. S., and Bond, H. E.: The resolution of mixtures of viable mammalian cells into homogeneous fractions by zonal centrifugation. J. Cell Biol 36: 369-378, 1968.

Serial No. NCI-4611
1. Biology Branch, OASDC,
   Etiology
2. Bioassay Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Metabolic Events in the Early Stages of Chemical Tumorigenesis

Previous Serial Number: Same

Principal Investigator: Richard R. Bates, M.D.

Other Investigators: Stuart H. Yuspa, M.D.

Cooperating Units: None

Man Years:
Total: 7.5
Professional: 2.0
Other: 5.5

Project Description

Objectives:

The purpose of this project is to determine the importance of specific metabolic activities in neoplastic transformation by chemical carcinogens.

Methods Employed:

The basic approach is twofold: to determine the effect of inhibitors of various metabolic activities on chemical carcinogenesis, and conversely, to examine the effect on normal cellular metabolism and the metabolism of chemical carcinogens of agents which stimulate or inhibit tumorigenesis. Most of these studies utilize the two-stage system of skin tumorigenesis. During the past year, we have studied the effect of gonadal function on skin tumorigenesis. Techniques for cell culture of epidermis are being developed for use in some of these studies.

Major Findings:

1. The NIH General Purpose male mouse is more susceptible than the female to skin tumorigenesis initiated by 7,12-dimethylbenz(a)anthracene (DMBA) and promoted by croton oil.

2. Castration before treatment with DMBA stimulates skin tumorigenesis in the females but results in a slight inhibition in the male.

3. Female mice in the metestrus or diestrus phases of the estrus cycle when DMBA is applied are more susceptible to skin tumorigenesis than those in proestrus or estrus.

Significance to Bio-Medical Research and the Program of the Institute:

Discovery that gonadal function affects skin tumorigenesis by DMBA provides a new procedure for analyzing metabolic activities required for chemical carcinogenesis. A comparison of the effect of hormones which stimulate tumorigenesis and those which inhibit tumorigenesis (on normal cell metabolism and metabolism of carcinogens) should give clues regarding the process of malignant transformation. A great deal of progress has been made in development of techniques for cell culture of epidermis. This tool will be very useful for studying *in vitro* carcinogenesis and the effects on it of metabolic inhibitors. This will permit a correlation of *in vitro* and *in vivo* studies using the same cell type but will avoid the penetration obstacle of the keratin layer *in vivo*.

Proposed Course:

The effect of various sex hormones on skin tumorigenesis is now being studied. When these experiments have resulted in a well-defined system yielding groups with high and low tumor yields, the effect of the hormones on nucleic acid and protein synthesis and on metabolism of carcinogens and their binding to macromolecules will be studied.

Publications

Richard R. Bates, Joseph S. Wortham, Wayne B. Counts, C. Wesley Dingman, and Harry V. Gelboin: Inhibition by Actinomycin D of DNA Synthesis and Skin Tumorigenesis Induced by 7,12-Dimethylbenz(a)anthracene. *Cancer Research* 28: 27-34, January 1968.

Serial No. NCI-4617
1. Biology Branch, OASDC, Etiology
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Carcinogen Screening Operations

Previous Serial Number: Same

Principal Investigators: E. K. Weisburger, Ph.D., and J. H. Weisburger, Ph.D.

Other Investigators: R. S. Yamamoto, Sc.D., R. M. Glass, M. D., and
H. H. Frankel, M. D.

Cooperating Units: None

Man Years

Total:	1.9
Professional:	1.2
Other:	0.7

Project Description

Objectives:

Studies are undertaken on the chronic effect or of the carcinogenicity of a number of chemicals of special interest or of mixtures of chemicals. The aim of these investigations is to gather information on hazards involved in handling certain chemicals or drugs and also on the mechanisms underlying joint effects in mixtures. Of concern also is the methodology of carcinogen screening with emphasis on improvement in sensitivity, speed and economy.

Methods Employed:

The chemicals are administered to a number of animal species by a variety of routes so as to secure optimal exposure of the animals and maximize the sensitivity of the test. The animals are observed and autopsied carefully. Data are secured by histopathological study of the tissues of experimental in comparison to control animals. In some instances related complementary investigations on the material, such as blood picture, metabolic pattern, and the like, are performed. It is hoped that new test systems will be a by-product of these investigations.

Major Findings:

1. Inhibition of toxicity and prevention of carcinogenicity of N-2-fluorenylacetamide (FAA) by acetanilide type compounds. As reported last year, acetanilide fed in the diet in a 50 molar excess inhibited the toxic reaction and prevented the carcinogenic effect of FAA in an experiment extending over 26 weeks. It has now been found that were these animals to be held for 52 weeks the survivors do show hepatoma. The tests demonstrate that the protection is partial, and probably competitive at the molecular level.

The isomeric ortho-, meta- and para-acetotoluidides also lowered the toxicity of FAA, but only the meta isomer prevented the carcinogenic effect. The para did not decrease carcinogenicity at all and the ortho isomer had an intermediary effect, whilst being toxic itself. Because the methyl group in the toluidine derivatives can be oxidized to the corresponding carboxy group, the effect of aminobenzoic acids in inhibiting toxicity and carcinogenicity of FAA were tested. Ortho-aminobenzoic acid failed to inhibit toxicity but the meta and para isomers did. Whether aminobenzoic acids prevent carcinogenicity will be known shortly.

Acetanilide prevented the carcinogenic effect of N-hydroxy-N-2-fluorenylacetamide, suggesting that the previously reported competitive inhibition of binding of active carcinogen metabolites by acetanilide may play a major role in the demonstrated effect.

As noted before, non-carcinogenic acetanilide or para-aminobenzoic acid might be recommended for therapeutic or prophylactic use in cases where accidental exposure to carcinogens of the aromatic amine type might have taken place.

2. Prevention of carcinogenicity of acetamide by arginine glutamate. Confirming an earlier report it was shown that the simple chemical compound of industrial importance, acetamide, was carcinogenic. In order to obtain information on the mode of action of this material, and at the same time arrive at a useful preventive treatment, tests were undertaken in which acetamide was fed to rats together with an equimolar amount of arginine glutamate. The underlying rationale was that acetamide could be considered carcinogenic because of intracellular liberation of excessive amounts of ammonium ions. The late Dr. Jesse Greenstein, and associates, NCI, have found that acute ammonia toxicity could be prevented by arginine glutamate. Our data now show that the carcinogenicity of acetamide can indeed be inhibited by non-toxic arginine glutamate, opening the door to further studies on the mechanism of action of acetamide.

Significance to Bio-medical Research and the Program of the Institute:

The study of the various factors involved in chemical carcinogenesis in respect to dose rate, synergism, the effect of species, target organ, and the examination of carcinogenicity of mutagenic and environmental agents fall within the framework of the responsibilities of the National Cancer Institute.

Proposed Course:

Many of the studies reported are long-term. Experiments will obviously lead to subsequent developments along the same lines.

Honors and Awards

Invited Lecture: J. H. Weisburger and J. DiPaolo: On Carcinogenic and Teratogenic Chemicals and Their Mode of Action. American Chemical Society (Medicinal Chemistry Division), Chicago, Illinois, September 10-15, 1967.

Invited Lecture: J. H. Weisburger: A Camera Looks at the Orient. ACS Meeting, Howard University, 1967.

Invited Lecture: J. H. Weisburger: Evaluation of Carcinogenicity of Drugs and other Chemicals. The APhA Academy of Pharmaceutical Sciences, Washington, D. C., November 26-28, 1967.

Invited Lecture: J. H. Weisburger: Problems of Drug Safety. Drug Research Board, National Academy of Sciences, Washington, D. C., March 20, 1968.

Member, Interdepartmental (FDA-USDA-PHS) Technical Panel on Carcinogens: J. H. Weisburger.

J. H. Weisburger: President, Washington Professional Chapter, Alpha Chi Sigma
Chairman, Public Relations Committee, Chemical Society of Washington
Honorary Editorial Advisory Board, *Food and Cosmetics Toxicology*
Board of Publications, *Toxicology and Applied Pharmacology*
Representative of American Association for Cancer Research to National Society for Medical Research

Publications

Yamamoto, R. S., Korzis, J., and Weisburger, J. H.: Chronic ethanol ingestion and the hepatocarcinogenicity of N-hydroxy-N-2-fluorenylacetamide. Int. J. Cancer 2: 337-43, 1967.

Weisburger, J. H., Hadidian, Z., Fredrickson, T. N., and Weisburger, E. K.: Host properties determine target, bladder or liver, in chemical carcinogenesis. In: Bladder Cancer, 5th Inter-American Conference on Toxicology and Occupational Medicine. W. Deichmann, K. Lampe, R. Penalver, and A. Soto, eds., Chapter 3, Aesculapius Publishing Co., Birmingham, Ala., 1967, pp. 45-57.

Weisburger, J. H., Weisburger, E. K., Griswold, D. P., and Casey, A. E.: Reduction of carcinogen-induced breast cancer in rats by an anti-fertility drug. Life Sciences 7: 259-66, 1968.

Griswold, D. P., Casey, A. E., Weisburger, E. K., and Weisburger, J. H.: The carcinogenicity of multiple intragastric doses of aromatic and heterocyclic nitro or amino derivatives in young female Sprague-Dawley rats. Cancer Res. (in press).

Weisburger, J. H., and Weisburger, E. K.: Food additives and chemical carcinogens: On the concept of zero tolerance. Food and Cosmetics Toxicol. (in press).

Tinsley, I. J., Harr, J. R., Bone, J. F., Weswig, P. H., and Yamamoto, R. S.: Selenium toxicity in rats. I. Growth and longevity. In Muth, C. (Ed.), Proc. Intern. Symposium on Selenium, Chapter 9, AVI Publishing Co., Westport, Conn., 1967, pp. 141-152.

Harr, J. R., Bone, J. F., Tinsley, I. J., Weswig, P. H., and Yamamoto, R. S.: Selenium toxicity in rats. II. Histopathology. In Muth, C. (Ed.), Proc. Intern. Symposium on Selenium, Chapter 10, AVI Publishing Co., Westport, Conn., 1967, pp. 153-178.

Serial No. NCI-4618
1. Biology Branch, OASDC, Etiology
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Mode of Action of Chemical Carcinogens -- Endogenous and Exogenous Factors in Chemical Carcinogenesis

Previous Serial Number: Same

Principal Investigator: J. H. Weisburger, Ph.D.

Other Investigators: R. S. Yamamoto, Sc.D., R. M. Glass, M. D., H. H. Frankel, M. D., Ph.D., E. K. Weisburger, Ph.D., and A. McCullough, M.S.

Cooperating Units: None

Man Years
Total: 5.6
Professional: 3.8
Other: 1.8

Project Description

Objectives:

Investigations to discover the intimate factors involved in the initiation and development of neoplasia are explored by means of animal experiments in various species. The studies utilized a number of chemical carcinogens administered to several species of animals under varying conditions of diet, endocrine situation or immunological status so as to gain insight into controlling mechanisms in the initiation and development of chemically induced tumors. Additional aims rest on the development of an in vitro system for the investigation of chemical carcinogenesis and its relationship to differentiation under simplified conditions.

Methods Employed:

Chemical carcinogens are given by several routes to a number of strains of rats on specific dietary regimens, and surgically or otherwise modified to yield definite endocrine states. In some cases, exogenous hormones are supplied. For some studies attempts are made to specifically immunize animals with tumor or tumor extracts. The animals are observed for the time required for neoplasms to appear. Careful autopsies are performed and the results are evaluated.

Utilizing published methods as background in vitro systems are investigated, specifically for the culture of parenchymal liver cells. The effect of hormonal factors and carcinogens under these conditions is receiving scrutiny.

Major Findings:

1. Effect of immunological situation in chemical carcinogenesis by N-2-fluorenylacetamide or the N-hydroxy derivative. The microsome fraction isolated by ultracentrifugation of liver tumors induced with FAA or the N-hydroxy derivative were used to "immunize" rats on a regular cycle of injections. Administration of the same carcinogens, utilizing our standard tumor induction regimen over a period of 26 weeks, induced hepatomas in rats pretreated with microsomes from liver tumor as well as in normal liver controls. The data show that, if present at all, the immunity conferred is very weak and can be overcome readily by moderately strong carcinogen stresses. Additional investigations will be necessary, utilizing a more moderate carcinogenic regimen.

With the idea that early cancer cells induced by a carcinogen are antigenic, experiments were designed to enhance carcinogenicity (and thereby render tests for carcinogens more sensitive) by lowering the immunological competence of animals. Preliminary experiments whereby the immunosuppressive drug azothioprine (Imuran) was given together with carcinogen, indicated that there was some enhancement, particularly with respect to toxic effects on the liver and the production of cirrhosis. Imuran itself exhibited some liver toxicity and at certain dose levels affected the general health of the rats, mainly in respect to enhanced pulmonary disease.

Along the same lines, tests were performed with antilymphocytic serum, a system used clinically to avoid rejection of organ transplants. Rats treated with antilymphocytic serum and carcinogen did very poorly. In addition, antilymphocytic serum itself gave rise to abnormalities in the liver and the spleen, as hepatosplenomegaly. The spleen showed loss of normal architecture and the white pulp areas almost disappeared with a replacement of the lymphocytes with reticulum type cells. In the liver there were degenerative or necrotic cells. Additional data are required to determine the specificity of the immune suppression seen in rats with respect to the effect of antilymphocytic serum. Control animals injected on the same chronic schedule with saline or normal rabbit serum continued to display normal chronologic development.

The effect of immunosuppressive agents on tumor homografts was studied utilizing hepatoma specific to Fischer rats injected into a number of other strains of rats. All strains completely rejected the tumor grafts with or without supplementary Imuran except for one. In the Sprague-Dawley strain the hepatoma grew in one experimental series in which Imuran had a potentiating effect and in another in which it did not. The Fischer tumors growing in Sprague-Dawley rats could be backcrossed into Fischer rats or continued in Sprague-Dawley rats. Additional studies with transplantable tumors to complement our investigations with primary induced tumors will be performed.

2. Development and utilization of liver tissue culture systems. In roller tube cultures on a collagen base rat and mouse liver explants have been grown satisfactorily. In a two- to three-week period there is cellular outgrowth with the characteristics of epithelial cells, rather than fibroblasts. Under our conditions, the cultures live at most for 3 to 4 weeks. Of a variety of chemical carcinogens placed in this system, the model carcinogen methylazoxymethanol acetate, an analog of the naturally occurring plant carcinogen cycasin, has permitted extended survival of epithelial cells. At this time several tubes with treated cultures are alive after over 10 months subsequent to a short exposure to methylazoxymethanol acetate. However, the roller tube culture system has a number of disadvantages, among which limited capability to secure a large number of cells is the most important. Thus, static cultures of rat, mouse and particularly hamster embryonic liver were established. In this case also, the life of the cultures is limited to about 3 or 4 weeks. Again, treatment with a variety of carcinogens at several dose levels failed to extend the life of the cultures, except when methylazoxymethanol acetate was utilized. After brief exposure to this agent there was active proliferation of parenchymal cells which developed some weeks later. These cells are now being subcultured and it is hoped sufficient amounts will be available for reimplantation into animals, as well as for biochemical studies.

Biochemical techniques were applied to establish that the cells growing out from the explants had the properties of parenchymal liver cells. In several instances it was demonstrated that radioactive leucine was incorporated by the cultures into a variety of proteins, one of which was specifically identified as serum albumin. Also, in a distinct test the cultured cells have been shown to possess the enzyme which can dehydroxylate N-hydroxy-N-2-fluorenylacetamide. These reactions appear to be specific for liver in mammalian systems.

Significance to Bio-medical Research and the Program of the Institute:

The prevention and cure of neoplasia hinges on an understanding of the intimate factors involved in the pathogenesis of cancer. The research performed within the framework of this report aims to produce data which will permit the comprehension of the underlying mechanisms involved in chemical carcinogenesis.

Proposed Course:

Various dietary, hormonal, and immunological factors will be investigated *in vivo* and *in vitro* for their effect in retarding or accelerating the development of induced neoplasia. It is hoped that the results obtained will not only give data of fundamental value but also produce better methods for the testing of chemical carcinogens. Specific short term approaches consist in further application of the *in vitro* liver culture system to study the carcinogenic potential of a series of compounds and to evaluate by biochemical techniques the underlying mechanism of any "transformation" obtained. Also, an important short term goal is to gather additional data relative to various conditions affecting immune status such as immunosuppressant drugs or antilymphocytic sera as well as the effect of the thymus in carcinogenesis.

Honors and Awards

Invited Lecture: J. H. Weisburger: Hepatic Carcinogenesis. International Symposium on Carcinogenesis and Carcinogen Testing, Boston, Massachusetts, November 8-9, 1967.

R. M. Glass: Visiting Lecturer in physiology, Catholic University

Publications

Weisburger, J. H.: Chemical carcinogenesis - A report on the 9th International Cancer Congress, Tokyo, Japan, October 23-29, 1966. *Cancer Res.* 27: 812-14, 1967.

Yamamoto, R. S., Weisburger, E. K., and Korzis, J.: Chronic administration of hydroxylamine and derivatives in mice. *Proc. Soc. Exptl. Biol. Med.* 124: 1217-20, 1967.

Weisburger, J. H. and Goodall, C. M.: Molecular pathology and carcinogenesis. *Science* 159: 115-116, 1968.

Weisburger, J. H.: Hormones, chemicals and liver cancer. *New Zealand Med. J.* 67: 44-58, 1968.

Banerjee, M. R. and Yamamoto, R. S.: Population of rat liver parenchymal cells with different patterns of ribonucleic acid synthesis during azo-dye carcinogenesis. *Cancer Res.* 28: 455-61, 1968.

Serial No. NCI-4619
1. Biology Branch, OASDC, Etiology
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Mode of Action of Chemical Carcinogens -- Studies on the Metabolism of Chemical Carcinogens

Previous Serial Number: Same

Principal Investigator: J. H. Weisburger, Ph.D.

Other Investigators: D. Szafarz, Ph.D., J. R. Williams, Jr., M. D., P. H. Grantham, M. S., and E. K. Weisburger, Ph.D.

Cooperating Units: Clinical Pathology Department, Clinical Center; Laboratory of Germ-free Animal Research, NIAID

Man Years

Total:	5.7
Professional:	4.4
Other:	1.3

Project Description

Objectives:

The aim of this research is to gather data relevant to the etiology of neoplasia at a molecular level. To this end chemical carcinogens, especially those of the aromatic amine type, are being utilized. Their metabolism and interaction with host tissues and specific targets are being studied. Simplified yet realistic model systems are devised in order to gain an understanding of the fundamental events operating in chemical carcinogenesis.

Methods Employed:

Biochemical and pharmacological techniques are applied to determine the metabolism of N-hydroxy-N-2-fluorenylacetamide (N-OH-FAA) and related compounds in various animal species. This includes isolation and purification of enzyme systems concerned with certain metabolic steps.

Procedures are developed for the separation, purification and analysis of the soluble and insoluble constituents of tissues of animals treated with chemical carcinogens, and of control animals.

Studies are performed in animals surgically modified to alter their hormonal balance, or with chemically or surgically damaged organs in order to investigate the effects of such conditions on the fate of the carcinogen.

The metabolism of chemical carcinogens and interaction with host targets is examined in the presence of other chemicals such as antibiotics or other molecular structures which affect the carcinogenic process. The aim of these experiments is to gain an understanding at the molecular level of inhibition or enhancement of carcinogenicity and thereby help to unravel the sequence of events leading to cancer.

Major Findings:

1. Immunochemical techniques applied to study of serum albumin and liver proteins. Anti-sera produced in rabbits to serum proteins of Fischer strain rats were used to show that during electrophoresis albumin from liver had a faster mobility than serum albumin. The reason for the increased mobility was the presence on liver albumin of materials with electronegative charge which could be removed by mild ether extraction or by treatment with absorbent charcoal. The treated albumin from liver had the same mobility as that from serum.

By electrophoretic and immunoelectrophoretic techniques liver tumors and liver were compared. In addition to alterations in the region of the β-globulins there were specific antigenic changes in respect to a select protein which was present in larger amounts in male than in female rats and which was almost absent in liver tumors. In addition, the electrophoretic technique as modified for the present purposes permitted quick identification of the "slow h_2 proteins" of Sorof. These proteins are important insofar as they show discrete changes during the carcinogenic process and are much reduced or indeed absent in liver tumors. These antigens have growth-controlling properties and may act as repressors.

Immunoelectrophoretic studies also revealed that rats treated with the immunosuppressant Imuran (see NCI-4618) exhibited a peculiar resolution of the serum γ-globulin bands into two arcs. The significance of this phenomenon is as yet unknown.

2. New metabolite of N-2-fluorenylacetamide and N-hydroxy-fluorenylacetamide in serum. Shortly after injection of N-2-fluorenylacetamide but more so with the N-hydroxy derivative the serum contained a new metabolite with a faster mobility on paper chromatograms than either of the precursors injected. This compound was shown to be the N-(3-methylthio-2-fluorenyl)-acetamide. The presence of this material in serum appears interesting because the mechanism of its formation may involve reaction either with methionine or more probably a methionine terminal group in a select protein.

3. Combination of carcinogen metabolites to molecular targets. One of the key contemporary problems in chemical carcinogenesis is the study of the receptors of active carcinogen metabolites. Whereas several carcinogens have been shown to bind to DNA, RNA and proteins, a direct connection between these demonstrated events and carcinogenesis remains to be made.

We have investigated the kinetics of combination of these essential structural and functional entities to permit possible selection of the crucial interaction. After a single injection of N-hydroxy-N-2-fluorenylacetamide, which under most conditions is not carcinogenic, appreciable binding to DNA, RNA and select nuclear, ribosomal, and microsomal proteins was observed. Under conditions where thymidine labeling of DNA was constant reflecting the stability of DNA, the attachment of carcinogen to DNA decreased progressively and reached unmeasurable values by day 12. The label attached to proteins in the supernatant and in the microsomes also declined and could not be detected after day 24. However, after continuing intake of N-hydroxy-N-2-fluorenylacetamide for 2 weeks, which may represent a weak carcinogenic regimen, the labeling on the proteins still declined at a rate similar to that seen after a single dose. However, after an initial rapid drop to day 8, the remaining binding to DNA was reasonably stable, with a half life of 17 days. A definite carcinogenic regimen of 10-week feeding is under investigation. The data thus far suggest that the carcinogen is attached more firmly to the genome and may thus affect its function for a prolonged period. Duplication of the altered DNA may be rendering such a transformation permanent, although additional data are required to substantiate fully this point.

4. Function of liver as affected by pituitary hormones and carcinogens. Earlier studies have shown that pituitary hormones not only potentiate liver carcinogenesis but also lead to tremendous hepatomegaly, and alterations in the serum proteins. Details of the latter aspect were investigated by studying plasma albumin synthesis in rats bearing a functional pituitary tumor giving rise to excessive hormone levels. Under these conditions the total production of albumin by liver is increased, particularly as regards the rate of albumin synthesis. Ribosomal RNA is likewise increased, but calculated as rate of albumin synthesis per unit ribosomal RNA, a decrease to 1/3 of normal is found. It is concluded that ribosomal RNA in animals with excess pituitary hormones is less efficient in the specific synthesis of serum albumin.

5. Metabolism of N-hydroxy-N-2-fluorenylacetamide and N-2-fluorenylacetamide in germ-free rats. Earlier studies have revealed that a sizable proportion of a dose of carcinogen is excreted in the bile of rats as the glucuronide of the N-hydroxy derivative. However, only a small portion eventually appears as this specific metabolite in urine. It was theorized as an explanation for the difference between bile and urinary metabolites that the gut flora probably participated in altering the glucuronide of N-hydroxy-N-2-fluorenylacetamide. A series of important studies, in cooperation with

the Clinical Pathology Branch of the Clinical Center and the Germ-free Laboratory of NIAID, have entirely confirmed that this is the case. Since germ-free rats without bacterial flora do not have the capability of converting this particular metabolite, they excreted it in sizable amounts in urine. By specific studies employing the cecum contents of conventional rats in comparison to the contents of germ-free rats it was unambiguously established that carcinogens such as N-2-fluorenylacetamide and the N-hydroxy derivative are metabolized to materials which can be acted upon by the enzyme β-glucuronidase and N-hydroxy-dehydroxylase provided by gut flora, particularly E. coli. An analysis of the metabolites present in feces likewise confirms the previous tests.

During continuing feeding of N-hydroxy-N-2-fluorenylacetamide, there is a progressive alteration of the bacterial flora in the gut with consequent changes in the metabolism of the carcinogen. Coliform organisms decrease and yeast type flora begins to predominate. It was believed heretofore that the changes in urinary metabolites seen during chronic intake of these agents were due to enzyme induction or deletion in liver. Our data unambiguously and for the first time involve the bacterial flora and its alterations during chronic feeding of the type of carcinogen in the changing picture of urinary metabolites.

In view of the increasing prolonged use of antibiotics which tend to drastically alter the bacterial flora, our findings call attention to the necessity to be aware of possible changes in drug action especially in cases where bacterial flora can be involved in drug metabolism.

Additionally, liver has been found to possess enzymes which dehydroxylate N-hydroxy-N-2-fluorenylacetamide. The enzyme is located in the soluble fraction of liver and can be concentrated by partial ammonium sulfate precipitation. These experiments will permit the eventual isolation of the important enzyme present in liver which controls, in part, the potency of carcinogens of the aromatic amine type. A better understanding of the elements which affect the level of this enzyme system may give clues to the prevention of the action of this class of agents.

Several of the above-named studies were facilitated by the development of a new and original method of measuring quantitatively the active metabolite N-hydroxy-N-2-fluorenylacetamide. The procedure consists in a brief oxidation by potassium permanganate to nitrosofluorene which is analyzed by an appropriate colorimetric reagent.

Significance to Bio-medical Research and the Program of the Institute:

An understanding of the etiology of neoplastic disease is of paramount importance in terms of the prevention and cure of the disease. Our various activities described in this report aim to shed light on the fundamental factors involved.

Proposed Course:

The program of elucidating the biochemistry of carcinogenesis, our eventual goal, is a continuing, long-term effort. Certain promising leads which have been discussed in this account will be followed up in further experiments.

Publications

Shirasu, Y., Grantham, P. H., Weisburger, E. K., and Weisburger, J. H.: Metabolism of continuously fed ^{14}C-labeled N-hydroxy-N-2-fluorenylacetamide at various intervals: Effect of pituitary hormones. Cancer Res. 27: 865-72, 1967.

Weisburger, E. K., Yamamoto, R. S., Glass, R. M., Grantham, P. H., and Weisburger, J. H.: Effect of neonatal androgen and estrogen injection on liver tumor induction by N-hydroxy-N-2-fluorenylacetamide and on the metabolism of carcinogen in rats. Endocrinology (in press).

Weisburger, J. H. and Goodall, C. M.: Steric inhibition of enzyme reactions. Lack of enzymic hydrolysis of 2',4',6'-trimethylacetanilide. Life Sciences (in press).

Serial No. NCI-4620
1. Biology Branch, OASDC, Etiology
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Mode of Action of Chemical Carcinogens - Chemical Investigations

Previous Serial Number: Same

Principal Investigator: E. K. Weisburger, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.8
Professional:	0.6
Other:	0.2

Project Description

Objectives:

In order to gain an understanding of the etiology of cancer, chemical studies are performed on the properties of certain carcinogens, and in model experiments, on the reaction of chemical carcinogens with select potential targets. The aim is to elucidate fundamental factors involved in chemical carcinogenesis.

Methods Employed:

Properties of chemicals acquired or synthesized for the bioassay of chemical carcinogens are investigated to determine the purity of the materials. Methods for purification and analysis of various chemicals are applied.

Major Findings:

The purity of compounds acquired or synthesized for long-term bioassay is checked. In addition samples of compounds were furnished to various investigators to further their research efforts, including: Dr. S. Epstein, Boston; Dr. J. Roth, Storrs; Dr. P. Newberne, Cambridge; Drs. M. Reuber and M. Anderson, NCI; Dr. C. M. Goodall, New Zealand; Dr. N. P. Buu-Hoi, France; and Dr. L. Bahna, Czechoslovakia.

Significance to Bio-medical Research and the Program of the Institute:

The studies performed are a necessary adjunct to the broad programs pursued in this Section.

Proposed Course:

As the biochemical and carcinogenesis experiments provide leads, model studies on the underlying chemistry will be performed. It is hoped that more emphasis can be placed on the chemical studies when the move to the new laboratory building is made.

Honors and Awards

E. K. Weisburger: Re-elected to the Board of Managers, Chemical Society of Washington, for 1968; also appointed to Election and Symposium Committees, Chemical Society of Washington.

E. K. Weisburger: Chairman, sub-group on "Chemicals," Discussion Group on Service Facilities and Reference Standards, NCI, June 8 and 9, 1967.

E. K. Weisburger: Board of Editos, Journal of the National Cancer Institute.

Publications

Thang, D. C., Weisburger, E. K., Mabille, Ph., and Buu-Hoi, N. P.: Carcinogenic nitrogen compounds. Part LVII. Hexacyclic acridines derived from phenanthrene and fluorene. J. Chem. Soc. 8(c): 665-68, 1967.

Suzuki, K. and Weisburger, E. K.: A reaction of nitrosobenzenes with aralkyl amines. J. Chem. Soc. 2(c): 199-202, 1968.

Serial No. NCI-4624
1. Biology Branch, OASDC, Etiology
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Study of carcinogens and other compounds as mutagens for *Salmonella typhimurium*

Previous Serial Number: Same

Principal Investigator: Laura C. Stewart, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.9
Professional:	0.9
Other:	0.0

Project Description

Objectives:

Many people believe that cancer is the result of a mutation induced in the genetic material of a cell and therefore carcinogens should exhibit mutagenic activity under the proper test conditions. This work was undertaken to try to establish a correlation between the carcinogenicity and mutagenicity of various compounds and if a positive correlation was established, to try to develop a simple rapid technique for the primary screening for carcinogenicity of the vast number of new chemicals constantly thrown into our environment.

Methods Employed:

Various types of histidine-requiring mutants of *Salmonella typhimurium* obtained from the Laboratory of Molecular Biology of the National Institutes of Health were used as test organisms. A total of 14 different mutants were tested. These can be classified into four principal types:

1. Mutants in which a phase shift has occurred in the genetic code by the addition or deletion of one or several (not three or a multiple of three) base pairs in the DNA.

2. Nonsense mutants (amber-ochre) in which the DNA has been altered to code for UAG or UAA in the messenger RNA. These triplets do not code for any amino acid but for breakage of the polypeptide chain and when this occurs before the end of the chain a defective protein is produced.

3. Missense type of mutants in which a base has been substituted for another in the DNA resulting in the incorporation of a different amino acid into the protein produced than the one originally coded for.

4. Large deletions in which a large segment of the chromosome has been lost.

The mutants were plated on minimal medium agar plates which will not support their growth unless a "reversion" to the wild type occurs. The chemicals to be tested were placed on the plates and the plates incubated for 36-48 hours at 37°. A mutagenic compound allows the organism to grow either by inducing a back mutation, a true reversion to the wild type, or more often by inducing the production of a working enzyme where in the original mutant only a defective enzyme or no enzyme at all could be produced.

Major Findings:

The major portion of the work involved testing peroxides since the Section is testing these compounds for carcinogenicity in mice.

By the usual procedure of placing several compounds on one bacterial plate these compounds did not show any or only questionable mutagenic activity. However, by setting up multiple identical platings (5 to 10) for each compound for each Salmonella mutant (one compound per plate) and comparing the number of "revertant" colonies obtained with spontaneous revertants on multiple blank platings, statistically significant results were obtained. The following peroxides were tested in this manner:

1. t-butyl hydroperoxide
2. di-t-butyl peroxide
3. t-butyl perbenzoate
4. t-butyl peracetate
5. ascaridole
6. cumene hydroperoxide
7. di-lauroyl peroxide
8. peroxylauric acid
9. peroxybenzoic acid
10. pelargonic peracid
11. 1,1'-dihydroxydicyclohexyl peroxide
12. dibenzoyl peroxide
13. dicumyl peroxide
14. p-nitroperoxybenzoic acid
15. 1-hydroxy-1'-hydroperoxydicyclohexyl perioxide
16. cyclohexane peroxycarboxylic acid
17. 2,6-di-t-butyl-4-t-butylperoxy-4-methylcyclohexa-2,5-diene-1-one
18. 2,5-dimethylhexane-2,5-dihydroperoxide
19. 2,6-di-t-butyl-4-hydroperoxy-4-methylcyclohexa-2,5-diene-1-one
20. 2,5-dimethyl-2,5-di-t-butylperoxy hexane

Volatile materials from heated corn oil which are believed to contain peroxy compounds were also tested.

Of the peroxides, t-butyl peracetate was mutagenic for seven of the fourteen mutants tested (B 14, C 117, C 151, C 340, C 527, D 130 and F 103). t-butyl hydroperoxide was slightly mutagenic for B 14, C 151, C 340 and more so for F 103. All the other peroxides were negative.

Several compounds prepared by Dr. Rice of this Section were tested. N-hydroxy urethane was slightly mutagenic for G 203 and D 130. Lysergic acid diethylamide (LSD) reported to produce chromosome breaks was tested and found to be negative.

Attempts to increase mutagenic response by pretreating the bacterial cells with ethylenediaminetetracetic acid (EDTA) or with dimethyl sulfoxide (DMSO) to alter the permeability of the cell membrane or by using caffeine or small doses of ultraviolet light to inhibit repair mechanisms did not enhance the effect for any of the compounds tested.

Proposed Course:

A positive correlation between carcinogenicity for mammals and mutagenicity for *Salmonella* of the many compounds tested during the three years this project was in progress, was obtained for only a few of the potent carcinogens tested such as strong alkylating agents. No correlation was demonstrated for possible weak carcinogens such as varicus insecticides (a slight response for captan was obtained), pesticides, herbicides, dyes, etc. It has, therefore, been decided to discontinue this project, although it may be possible that in some other test system such a correlation might be established. However, the data on the mutagenic activity of the peroxide will be evaluated in the light of other experiments being conducted by the Section with these compounds, and a short note prepared for publication.

Serial No. NCI-4625
1. Biology Branch, OASDC, Etiology
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Carcinogenicity of heated fats

Previous Serial Number: Same

Principal Investigator: Laura C. Stewart, Ph.D.

Other Investigators: Roger O'Gara, M.D.

Cooperating Units: Laboratory of Pathology, NCI (see NCI-5266)

Man Years:
Total: 0.1
Professional: 0.1
Other: 0.0

Project Description

Objectives:

Fats have, for a long time, been suspected of developing carcinogenic properties during the heating process, especially during repeated heating at elevated temperatures, such as is done in commercial and home deep-fat frying. The results reported in the literature of laboratory experiments to test this hypothesis are inconclusive and, at times, contradictory. This work was undertaken in the hope of contributing some information to this problem. The experiments to test the carcinogenicity of heated fats started in 1963 in the Environmental Cancer Section and are being continued with the cooperation of Dr. Roger O'Gara of the Pathology Department of NCI.

Methods Employed:

Two kinds of fat were tested: (1) Used discarded fat from the Clinical Center cafeteria. This is a hydrogenated vegetable oil, solid at room temperature, with an iodine number of 73 for the fresh unheated fat, which was used as control, and of 71 for the used fat. (2) Corn oil which was heated in the laboratory in a small commercial type fryer. The heating was carried out in lots of 8 liters at 195° ± 8° (390 F. a temperature frequently used in commercial and home frying). Air was blown through the oil by means of a fritted glass gas dispersion tube at the rate of 250 cc air/min/liter to simulate the stirring that occurs during the actual frying of foods. Potatoes were fried occasionally to introduce moisture into the oil. The oil was heated 6 or 7 hours daily for a total of 48-50 hours, or until a refractive index of 1.4830 and an iodine number of 87 were reached. The fresh unheated oil used as control has a refractive index of 1.4730 and an iodine number of 119.6.

Three fractions were prepared from each fat as follows:

1. The unsaponifiable fraction which is principally the steroids naturally occurring in the fat, plus any hydrocarbons which might have developed through hydrolysis of the fat and decarboxylation of the free fatty acids during the heating process.

2. The urea adduct fraction which consists of the unchanged straight chain fatty acids. This is composed principally of the saturated fatty acids which were present in the original fat, and is solid at room temperature even when obtained from corn oil.

3. The non-urea adduct fraction. This consists of the fatty acids which were changed during the heating process, through cyclization and especially through polymerization. The unsaturated fatty acids are those principally involved in these transformations which might involve carbon to carbon polymerizations or carbon to oxygen condensations of oxidation products of the fatty acids.

In addition to these, from the corn oil only, a fourth fraction, the volatile material produced during heating, was prepared.

All fractions were tested on NIH black rats by subcutaneous injections into newborns, bimonthly intramuscular injections into weanlings, and by feeding. For some fractions, such as the non-urea adduct of heated corn oil, all three routes of administration were being employed. For some, such as the volatiles, the unsaponifiables, and the non-urea adduct of the fresh and used Clinical Center cafeteria fats, where the yields are so low that it is impractical to prepare sufficient amounts for feeding, only the injection methods were used. In addition, the cafeteria fats were tested by feeding the whole fat.

The feeding experiment was set up as follows: Five diets were prepared using ground rat pellets into which the fat was incorporated in the following amounts:

20% used cafeteria fat
20% new cafeteria fat
10% fresh corn oil
5% urea adduct of heated corn oil plus 5% fresh corn oil
5% non-urea adduct of heated corn oil plus 5% fresh corn oil

A vitamin supplement of 2000 units vitamin A, 400 units vitamin D, and 5 mg vitamin E was given each rat weekly. A preliminary study showed that the rats tolerated these diets well and made satisfactory growth gains. 100

animals, 40 males and 60 females, were on each diet. Half of the animals on each diet were given, orally, a small amount of 3,4-benzpyrene, 0.5 mg per rat per week, to see if the heated fat has any synergistic effect with a know carcinogen. Forty (40) to 70 animals were used for injections of each fraction.

A total of 1,600 rats were used in this study. Routine care of the animals was under contract with Microbiological Associates, Inc. from July 1964 until the last of the animals were sacrificed in October 1965.

Major Findings:

Dr. Roger O'Gara has completed the pathological and histological examination and is evaluating the results. There appears to be a carcinogen in the non-urea adduct forming fraction (NUA) of fresh fats, both of the saturated Clinical Center cafeteria fat and of the polyunsaturated corn oil. 5.9% and 13.5% respectively of the rats receiving these fractions as biweekly intramuscular injections developed tumors. This carcinogenicity was apparently increased 3 to 4 fold by the repeated heating of the fats, 19.5% of the animals injected with NUA of used cafeteria fat and 59.3% of those injected with NUA of the laboratory heated corn oil developed tumors. None of the control animals injected with whole fresh corn oil developed any tumors.

In the feeding experiments oral administration of a small amount, 0.5 mg/rat/week, of 3,4-benzpyrene per os increased the carcinogenic response of all the fat fractions. The urea adduct of heated corn oil when fed without the benzpyrene also produced a slight increase of tumors. Unfortunately, due to circumstances beyond our control, so many of the animals on the non-urea adduct of the heated corn oil died early in the experiment that no meaningful results can be reported for this fraction.

The animals injected subcutaneously as newborns did not show any significant increase in tumors over the controls.

Significance to Bio-medical Research and the Program of the Institute:

It is extremely important to determine if fats acquire carcinogenic properties on repeated heating, since deep fat frying, a procedure in which the fat is heated over and over, with addition of fresh fat as it is needed to replace that removed by the food, is a very popular and frequently used method in the preparation of food for human consumption.

Proposed Course:

The data have been evaluated and are being prepared for publication.

Serial No. NCI-4628
1. Biology Branch, OASDC, Etiology
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Biochemical effect of thalidomide and derivatives

Previous Serial Number: Same

Principal Investigator: Joseph A. DiPaolo, Ph.D.

Other Investigators: John S. Wolff, III, Ph.D.

Cooperating Units: None

Man Years:
Total: 0.1
Professional: 0.05
Other: 0.05

Project Description

Objectives:

To determine if thalidomide or its aqueous degradation products would form any type of chemical or physical association with human chromosomes.

Methods Employed:

Since we were unsuccessful in demonstrating alteration in nucleic acids by thalidomide in enzyme free systems, we have turned to short-term cultures of peripheral leukocytes in McCoy's medium 5a supplemented with fetal calf serum. DNA replication of chromosomes by the use of radioactive thalidomide is being introduced at different times during the culture period to determine the extent of binding to chromosomes.

Major Findings:

No chromosome breaks have been associated with thalidomide. Studies concerning binding are incomplete. It appears that thalidomide inhibits DNA of human lymphocytes.

Significance to Bio-medical Research and the Program of the Institute:

Study of this compound is related to cancer research since there is a growing realization that relationships exist between oncology and teratology.

Proposed Course:

After these experiments described above are completed the project will be considered closed.

Serial No. NCI-4629
1. Biology Branch, OASDC, Etiology
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The mechanism of cell transformation

Previous Serial Number: Same

Principal Investigator: Joseph A. DiPaolo, Ph.D.

Other Investigators: Lawrence J. Alfred, Ph.D. and Alan Rabson, M.D.

Cooperating Units: Pathologic Anatomy Branch, General Laboratories and Clinics, NCI

Man Years:
Total: 4.5
Professional: 2.5
Other: 2.0

Project Description

Objectives:

A determination of some aspects of neoplastic cell transformation *in vitro* with special reference to nucleic acid biosynthesis and population dynamics. The long term aim of this project is the systematic characterization of the affect on chemical carcinogens and non-carcinogens during neoplastic development by the use of nucleic acid biosynthetic mechanisms and cellular biology.

Methods Employed:

Mass cultures resulting from trypsinized whole Syrian hamster embryos are exposed to carcinogenic and non-carcinogenic polycyclic hydrocarbons and analyzed for their ability to produce tumors. Synchronized cells are also employed to determine if a specific phase in the cell cycle is involved in initial carcinogen-cell interaction. DNA isolation by cesium chloride density equilibrium is used to study the binding of carcinogens alone and in combination with other types of compounds. Radioautographic analysis is used to correlate the biochemical effects with cellular events on intact cells.

Major Findings:

Protection is defined as the ability of BA treatment to prevent DMBA-initiated cell lysis, as well as inhibition of cell division and DNA synthesis. The induction of the microsomal bound detoxifying enzyme system, benzpyrene hydroxylase (BP OH-lase), by these carcinogens was also studied. Secondary cultures, blocked in the S period with excess thymidine (5-7.5mm) were treated with 2-4μg BA, DMBA, or BA + DMBA/ml culture medium during suppression and resumption of DNA synthesis. Cells reversed from thymidine blockage, and labeled with deoxcytidine-3H (CdR) and thymidine-2^{14}C (Tdr) simultaneously, incorporated CdR into DNA maximally during the first hour, but the uptake of Tdr began 1 hour later. Hydrocarbon treatment had no effect on CdR incorporation, but DMBA suppressed the rate of incorporation of Tdr into DNA (50% of control after 2 hours). DMBA inhibition of DNA synthesis and cell lysis were almost completely prevented by simultaneous BA treatment. Induction of BP OH-lase activity after reversal of thymidine blockage reached a maximum at 4 hours, and declined after development of contact inhibition (72 hours). Addition of fresh inducer produced a reinduction. Enzyme activity was higher during complete suppression (0.4% cells in synthesis) than after resumption of DNA synthesis. Thus, induction occurs primarily during the G1 period of the mitotic cycle, where protection against cytotoxicity and cell lysis is probably initiated.

Significance to Bio-medical Research and the Program of the Institute:

Carcinogenesis is a phenomenon well suited to analysis in isolated cell systems. Since there is evidence that neoplastic transformation is a problem in population dynamics or ecology the study of loss of growth control and demonstration of tumor producing ability as shown by inoculation into appropriate hosts should permit a study of those events at both the initial period of insult as well as at the time of transformation.

Proposed Course:

It is planned to study the mechanisms of early events of carcinogen cell interaction. In addition experiments are planned to test whether or not neoplastic transformation has occurred as a result of the chemical used, was due to selection, or due to viral activation. The tumorigenicity of cells exposed for different time intervals will be determined.

Honors and Awards

Lecture, Institute for Cancer Research, Philadelphia, Pennsylvania, 1967, Joseph A. DiPaolo.

Lecture, New York University, New York, New York, 1967, Joseph A. DiPaolo.

Publications

Alfred, L. J. and DiPaolo, J. A.: Reversible inhibition of DNA synthesis in hamster embryo cells in culture: action of 1,2-Benzanthracene and 7,12-Dimethylbenz(a)anthracene. Cancer Research 28: 60-65, 1968.

DiPaolo, J. A.: *In vitro* spontaneous neoplastic transformation of mouse heart tissue. Nature 213: 932-933, 1967.

DiPaolo, J. A. and Banerjee, M.: Autoradiographic demonstration of H^3 methylcholanthrene labeling to nuclear components of Syrian hamster cells *in vitro*. *Proc. Natl. Acad. Sci.* 58: 123-126, 1967.

DiPaolo, J. A. and Donovan, P. J.: Properties of Syrian hamster cells transformed in the presence of carcinogenic hydrocarbons. *Expl. Cell Research* 48: 361-377, 1967.

Serial No. NCI-4631
1. Biology Branch, OASDC, Etiology
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Experimental mammalian teratology and its relationship to carcinogenesis

Previous Serial Number: Same

Principal Investigator: Joseph A. DiPaolo, Ph.D.

Other Investigators: Harry M. Givelber, M.D.

Cooperating Units: None

Man Years:
Total: 1.0
Professional: 0.6
Other: 0.4

Project Description

Objectives:

To determine the extent to which carcinogenic compounds are teratogenic and the extent to which teratogenic compounds are carcinogenic. To develop an *in vitro* system for study of teratogenesis using the 8 day hamster embryo in organ culture. Using this method one could separate the direct teratogenic effects of compounds as opposed to indirect maternal effects. To determine whether the teratogenicity can be used as an index of carcinogenicity, and therefore whether a short-term teratogenic experiment can be used to predict which compounds should be studied for long-term carcinogenesis. Finally to study the mechanism of action of teratogenic compounds and see the relation (if any) to the mechanisms of carcinogenesis.

Methods Employed:

Mice and hamsters are used in the studies. Careful record is kept of the gestational stage at which the teratogen is applied so that critical periods are established. Both single and multiple generation reproduction studies are being made to evaluate fertility of male and female, ova implantation, embryonic and fetal development, resorption, abortion, delivery, live births, litter size, teratogenic effects (both macroscopic and microscopic), viability of newborn, maturation of young, and nutritional and toxic aspects of mothers' milk. Entire embryos are being cultured for studying the direct effects of teratogens and carcinogens.

Serial No. NCI-4631

Major Findings:

A number of liquid organic peroxides (dibenzoyl peroxide, pelargonic peroxide, peroxybenzoic acid, p-nitroperoxybenzoic acid, dihydroxycyclobenzyl peroxide and 1-hydroxy-1'-hydroperoxydicyclohexyl peroxide) have been tested and shown to be non-teratogenic under the above conditions. Investigation of other urethane derivatives including p-nitrobenzenesulfonoxy-urethane, acetoxyurethane, N-methyl N-nitrosourethane and N-ethyl N-nitrosourethane has shown that some of these compounds are quite embryotoxic and often toxic to the pregnant hamster as well at relatively low doses (0.25 mM/kg body weight), but congenital malformations were not found. Viruses studied including RSV, Polyoma, Coxsackie B1, Adenovirus 12, H1 virus and EMC virus are all non-teratogenic, though the H1 and EMC viruses are quite embryotoxic at considerable dilutions. The EMC virus in addition causes posterior paralysis and death in the pregnant hamster at dilutions of 10^{-9} of the pooled virus.

Experiments with LSD in both single and multiple injections indicate that contrary to published reports of Auerbach and Rugowski, and Geber, but in agreement with Warkany this compound is not teratogenic.

Preliminary work on Actinomycin D suggests that this compound is teratogenic in the hamster.

Ethylnitrosourea is strongly teratogenic at a dose of 0.5 mM/kg body weight producing at sacrifice on day 15 malformations at 41 per cent of the total sites, or 90 per cent of the total live fetuses. These malformations are mainly of the CNS, eye or limbs. If animals are sacrificed on days 11 through 15 (after injection on day 8) there is:

1. a change in the type of malformation with severe cardiovascular and CNS malformations appearing on day 11, but not observed afterwards;

2. a generally inverse relationship between the percentage of resorptions and the percentage of live fetuses or the percentage of malformations with increasing time after injection;

3. an increase in the percentage of live fetuses with malformations with increasing time after injection.

Using 30 per cent hamster serum and 70 per cent McCoy's 5a medium, and changing the medium every 24 hours, a majority of the embryos can be kept alive from 40 to 48 hours after transplantation with beating hearts and intact circulation. Maximum development includes the appearance of anterior and posterior limb buds. However, a significant proportion of the embryos have a malformation involving the cardiovascular system with precordial edema and a greatly enlarged pericardium. Other malformations include twisting of the spine and anencephaly. These malformations are similar to those fetal malformations seen *in vivo* at 10 or 11 days after administration of ethylnitrosourea on day 8. In terms of growth the protein has increased two to five times in those fetuses surviving 48 hours *in vitro*.

Significance to Bio-medical Research and the Program of the Institute:

While there is evidence both clinical and experimental of a relationship between teratogenesis and carcinogenesis, the current work indicates that a carcinogen is not necessarily a teratogen, at least under the conditions of a single injection on day 8 in the hamster. When the two qualities are combined in the same compound as in ethylnitrosourea, it is of some interest whether the mode of action is similar. The wide spectrum of malformations induced with ethylnitrosourea indicates that it is probably interfering with basic cellular functions rather than interfering with the specific development of a specific organ (compare for example the action of hydrocortisone and the development of cleft palate in mice). Detailed study of selected cases such as ethylnitrosourea will probably be more fruitful than mass testing of carcinogens and teratogens.

A method of *in vitro* culture for whole embryos would allow detailed study of development without the complicating maternal factors, as well as allowing direct observation of the effects of teratogens. A start toward this objective has been made, but more study is required to define the factors that are causing malformations under the present conditions.

Proposed Course:

Further analogues of carcinogenic compounds especially of N-ethylnitrosourea, will be studied. The role of these compounds as diazoalkyl generators is of special interest. In addition other potential carcinogens will be studied for their teratogenic effect using mice and hamsters.

Attempts will be made to prolong development of embryos in culture by alterations of the medium atmosphere. Once optimum conditions of culture are determined, study of chemical alterations of development can begin.

Publications

DiPaolo, J. A.: A perspective of teratology and oncology. *Congenital Anomalies* 7: 61-63, 1967.

DiPaolo, J. A.: LSD; effects on offspring. *Science* 158: 522, 1967.

DiPaolo, J. A. and Elis, J.: The comparison of teratogenic and carcinogenic effects of some carbamate compounds. *Cancer Research* 27: 1696-1701, 1967.

DiPaolo, J. A. and Elis, J. and Erwin, H.: The teratogenic response by different species to aflatoxin B_1. *Nature* 215: 638-639, 1967.

Serial No. NCI-4643
1. Biology Branch, OASDC, Etiology
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Interrelationship of macromolecular events in the cell cycle studied in cultured human peripheral blood lymphocytes

Previous Serial Number: Same

Principal Investigator: J. S. Wolff, III, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:	
Total:	2.0
Professional:	2.0
Other:	0.0

Project Description

Objectives:

To determine the relation between various macromolecular synthetic events in cultured human peripheral blood lymphocytes exposed to a mitogenic stimulus in order to define the cell cycle. To elucidate the mechanism of mitogenic stimulation and transformation.

Methods Employed:

Cell culture techniques, protein and cell fractionation, chromatography, autoradiography, radioactive counting, precursor incorporation, and electrophoreisis are used in this project.

Major Findings:

Deoxyribonucleic acid synthesis, and subsequent mitosis, in cultured human peripheral blood lymphocytes exposed to a mitogenic stimulus is dependent on prior synthesis of ribonucleic acid and protein. In fact, DNA synthesis is dependent on RNA and protein synthesis up to within 30 minutes of its beginning, in these cells, as shown by inhibitor studies.

When lymphocytes are exposed to two mitogens (e.g. phytohemagglutinin and pokeweed), the response, in terms of DNA synthesis and cell division, is increased over that obtained with a single mitogen. That the response is not additive suggests that some cells possess at least a dual response potential.

The mechanism of mitogenic/stimulation is under study with the use of a previously characterized DNA-dependent RNA polymerase in nuclei of human peripheral blood lymphocytes. Evidence to date suggests that there is an immediate increase in the activity of this enzyme system. The nature of this increase is now under study.

Significance to Bio-medical Research and the Program of the Institute:

The determination of the mechanics of operation and control in a system of normal cells (lymphocytes) is a prerequisite to the determination of anomalies in abnormal cells (in this case leukemic lymphocytes).

Proposed Course:

For the remainder of this fiscal year, the effects of metabolic inhibitors and teratogens on macromolecular synthesis will be studied in cultures of human peripheral blood lymphocytes. Further attempts will be made to elucidate the nature of the control mechanism in these cells.

Serial No. NCI-4645
1. Biology Branch, OASDC, Etiology
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Induction of temperate bacteriophages by carcinogens

Previous Serial Number: Same

Principal Investigator: Marvin D. Anderson, M.D.

Other Investigators: Joseph A. DiPaolo, Ph.D. and Hans L. Falk, Ph.D.

Cooperating Units: None

Man Years:
Total: 1.0
Professional: 1.0
Other: 0.0

Project Description

Objectives:

Purposes include development and use of a simple, rapid test to detect suspected chemical carcinogens, and to investigate the biochemical mode of action of compounds that are carcinogenic in mammals.

Methods Employed:

Lysogenic bacterial strains growing in defined media are exposed to suspected inducting agents. After incubation to allow phage maturation and cell lysis, both viruses and uninduced bacteria are assayed. The criterion of induction is based upon plaque yields of treated versus control cultures. Determination of minimal inducing doses of active compounds permits their comparison and investigation of their mode of action.

Major Findings:

Dimethyl Sulfoxide (DMSO) allowed solubilization and examination of non-polar carcinogens. Hence water-solubility of compounds was not prerequisite in this system. Generally, parent compounds were inactive, but some metabolites which, unlike the parent compound, were potent local carcinogens and were therefore, suspected of being proximate carcinogens induced the bacterial virus. All positive compounds were either N-hydroxylated or nitroso derivatives, and most have been shown to be formed in vivo by the susceptible species. Positive results were obtained with N-hydroxyurethan, N-methyl-and N-ethyl-N-nitrosoureas and -urethanes, alpha and beta hydroxylaminonaphthalenes and 2-nitrosofluorene.

Significance to Bio-medical Research and the Program of the Institute:

Bacteriophage induction offers a simple microbiological model for studying compounds which are carcinogenic to mammals. Its selectivity for suspected proximate carcinogens offers a means for their detection as well as possibilities for studying their mode of action. Microgram sensitivity permits testing of even rare, expensive compounds. As a pilot study, phage induction can indicate likely compounds for carcinogenicity testing in mammals. Finally, the emphasis this system provides on the importance of supplying proximate carcinogens may improve results from *in vitro* transformation

Proposed Course:

For the balance of this fiscal year, further study of proximate carcinogens is projected. Emphasis will be placed on determination of common structural properties. Attempts to investigate classic polycyclic aromatic carcinogens will continue.

Publications

Anderson, M. D. and DiPaolo, J. A.: Lamba (λ) bacteriophage inducing capability of some chemical carcinogens. *Fed. Proc.* 27: 607 (Abstract No. 2204), March-April, 1968.

Anderson, M. D. and DiPaolo, J. A.: Detection of proximate chemical carcinogens by bacteriophage induction. *Science*, 1968.

Serial No. NCI-4649
1. Biology Branch, OASDC, Etiology
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on known carcinogens and potential environmental carcinogens

Previous Serial Number: None

Principal Investigator: Joseph A. DiPaolo, Ph.D.

Other Investigators: Harry Givelber, M.D., Paul Kotin, M.D., Hans L. Falk, Ph.D. and Marvin Schneiderman, Ph.D.

Cooperating Units: Biometry Branch, Demography Area, NCI

Man Years:
Total: 1.0
Professional: 0.5
Other: 0.5

Project Description

Objectives:

To study the carcinogenic effect of a wide variety of chemical agents which may represent new classes of carcinogenesis, cocarcinogens or anticarcinogens. To use a wide spectrum of mouse strains and ages (neonatal and mature) in order to increase the possibility of discerning carcinogenic response.

Methods Employed:

Mice are treated orally, subcutaneously or intraperitoneally with single or multiple doses of chemicals. After several weeks of treatment the mice are set aside for observation. Depending upon the compound the animals are killed at different intervals and autopsied.

Major Findings:

The RF/Un mice known to respond to radiation with development of thymic lymphomas and granulocytic leukemia were treated with 300 R and Actinomycin in combination and separately. As measured by survival analysis, Actinomycin D alone did not change either the incidence of lymphomas and/or leukemia, or the overall survival rates. In combination with X-radiation, Actinomycin D was far more toxic in male than in female mice, but had no effect on survival rate or tumor incidence.

Animal studies have been successful in clarifying confusion over structure and carcinogenicity. We have confirmed that both dibenzo [a, l] pyrene and the compound previously assigned that name in error, dibenzo [a, e] flouranthene, are carcinogenic to the subcutaneous tissues of C57Bl/6 mice.

Carcinogenesis experiments are underway using pure compounds belonging to a broad spectrum of peroxide and lactone structures provided by Dr. Swern under a contract with Fels Research Institute. Although tumors have not been observed as yet, the differential toxicity noted has required change in drug regime and the addition of new animals to many groups.

Significance to Bio-medical Research and the Program of the Institute:

Organic peroxides which are known to be generated in cooking oils or to have radiomimetic effects should be studied for carcinogenicity.

Studies on cuting oils or asbestos that is used in heart surgery must be studied to determine whether or not they represent potential hazards to humans.

Proposed Course:

Not definite yet.

Publications

Givelber, H., DiPaolo, J. A. and Schneiderman, M.: The influence of Actinomycin D on survival of X-radiated RF mice. Cancer Research, 1968 (In Press).

Serial No. NCI-4650
1. Biology Branch, OASDC, Etiology
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Transplacental carcinogenesis in mice by ethylnitrosourea and related compounds

Previous Serial Number: None

Principal Investigator: Jerry M. Rice, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 2.0
Professional: 1.0
Other: 1.0

Project Description

Objectives:

To ascertain the conditions under which tumors may be induced in mice during fetal life by treatment of the mother with selected chemical carcinogens, and to evaluate the effect of immunological and endocrine factors on the number and kinds of tumors observed.

Methods Employed:

Most of the carcinogens are not commercially available, and were synthesized. Pregnant mice of strains A, C3HfB, C57Bl/6 and NIH General Purpose were injected on specified days during gestation with solutions of the carcinogen in tricaprylin. The offspring were autopsied at ages of 12 to 40 weeks.

Major Findings:

It was first reported by Druckrey that ethylnitrosourea specifically induces tumors of the brain and cranial nerves when administered transplacentally to rats. We find that mice exposed to a single dose of this compound *in utero* develop thymic lymphomas and a high yield of multiple pulmonary adenomas and hepatomas, as well as a less striking incidence of other tumors. No tumors of the central nervous system have been seen in mice. The major generalizations derived from our experiments include the following:

1. Pulmonary tumors can be induced in mice as early as the 12th day of gestation, and (with increasing tumor yield) at any period between this time and a few hours prior to parturition.

2. Ethylnitrosourea is maximally effective in inducing pulmonary tumors when administered on day 16-17 of gestation, which terminates on the 19th day for inbred strains and on the 21st day for general purpose mice. This is the period when lymphoid proliferation in the thymus is most rapid, so the possibility exists that its immunosuppressive activity constitutes an important aspect of the carcinogenic potency of this compound. The ability of carcinogenic doses of ethylnitrosourea to prevent allograft rejection in adult mice is under study.

3. Adults of the various strains of mice used in these studies differ considerably in their susceptibility to induction of lung and liver tumors and lymphomas: general purpose mice are highly susceptible to thymic lymphomas; general purpose and strain A mice are very sensitive to pulmonary adenoma induction, while C3Hf's are moderately resistant and C57Bl/6's are extremely resistant; C3Hf mice are very sensitive to hepatoma induction, and strain A's moderately so. Transplacental exposure to comparable amounts of ethylnitrosourea at equivalent stages in pregnancy, however, gives quantitatively the same pulmonary adenoma response in all strains of mice tested. Whatever the factors governing resistance to these tumors are, they seem to develop after birth. This observation does not extend to liver tumors or lymphomas.

4. Hepatomas induced transplacentally between the 14th and 19th days of pregnancy have much longer latent periods than the other types of tumor, and exhibit a pronounced sex affinity: males develop tumors earlier and in much greater numbers than females. It seems likely that this effect is mediated by sex hormones, and has nothing to do with either penetration of the carcinogen into liver cells or a differential effect on metabolism or detoxifixation of the carcinogen, since exposure to the latter occurred _in utero_. Studies on the effects of gonadectomy and hormone replacement therapy on the development of these tumors in C3Hf mice are in progress.

5. Doses of ethylnitrosourea which are too small to elicit a significant pulmonary tumor or lymphoma response in adult general purpose mice nevertheless induce both types of tumor in the offspring of females treated at appropriate times during pregnancy.

Significance to Bio-medical Research and the Program of the Institute:

The demonstration that an optimum period for the transplacental induction of pulmonary tumors does exist in mice, and that it corresponds to the time late in pregnancy when lymphoid differentiation is occurring in the thymus, contrasts with the well-known fact that malformations are most likely to occur early in pregnancy. The sex difference in fetal susceptibility to hepatoma induction seen in offspring of treated C3H mice is most likely due to "promotion" by male sex hormones of development of tumors from latent transformed cells.

Serial No. NCI-4650

Proposed Course:

The immunosuppressive effect of ethylnitrosourea and related compounds should be investigated. The nature of the sex effect on hepatoma development should be explored. The pharmacology of these carcinogens should be investigated using radioactive tracer methods to define their course through the tissues of treated mice.

Serial No. NCI-4636
1. Biology Branch, OASDC, Etiology
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Physico Chemical Characteristics of Complement Components

Previous Serial Number: Same

Principal Investigator: Harvey R. Colten, M. D.

Other Investigators: Tibor Borsos, Sc.D. and Herbert J. Rapp, Sc.D.

Cooperating Units: None

Man Years

Total:	2
Professional:	1
Other:	1

Project Description

Objectives:

To isolate chemically and functionally pure serum complement components and to characterize the physico-chemical properties and structure function relationships of these biologically active molecules.

Methods Employed:

Complement components are purified by a number of techniques including gel filtration, preparative ultracentrifugation (including the B XIV zonal ultracentrifuge), ion exchange chromatography and a new technique for the separation of molecules based differences in isoelectric point. The effects on structure and function of isolated complement components of a number of physico-chemical parameters, such as changes in ionic strength, trace metal content, temperature and osmolarity are studied with immunochemical and physico-chemical techniques.

Major Findings:

We have found that the first component of complement C'1 is reversibly dissociated as a function of ionic strength. This dissociation leads to a corresponding change in sedimentation rate as a function of ionic strength. Methods based on this finding are being used to prepare highly purified C'1. Briefly, preparative ultracentrifugation of a euglobulin fraction of serum at different ionic strengths has resulted in purified C'1 preparations in which up to 85% of the protein is biologically active C'1.

Use of a new method of separating proteins on the basis of isoelectric point has revealed the presence of two forms of the second component of complement (C'2). These two forms are functionally identical and under the conditions of this method differ in isoelectric point by less than 0.25 pH units. These probably represent isozymes of the C'2 molecule.

Significance to Bio-medical Research and the Program of the Institute:

Basic studies of chemical and functional relationships of complement components are directed toward an understanding of the mechanism of complement fixation in general, and have potential application to studies of mechanisms of host resistance to infectious disease and tumors. Specifically it is necessary to determine the factors which inhibit or enhance the immunologic response to antigens. In the case of complement mediated cytotoxic reactions, isolation of cytotoxic antibody, cell antigens and complement in chemically or functionally pure form permits study of controlled reactions in which an estimate of the importance of a variety of chemical and physical conditions can be made. The detection of isozymic forms of complement components provides a genetic marker for a protein participating in immune cytotoxic reactions.

Serial No. NCI-4636

Proposed Course:

Functional immunochemical and physico chemical characterization of highly purified C'1 preparations continues. Furthermore, investigation of the isozymes of C'2 in individual guinea pigs is now in progress.

Publications

Colten, H. R., Borsos, T., and Rapp, H. J.: Reversible loss of activity of the first component of complement (C'1) as a function of ionic strength. J. Immun., 100: 799-807, April, 1968

Colten, H. R., Borsos, T., and Rapp, H. J.: Ultracentrifugation of the first component of complement: Effects of ionic strength. J. Immun., 100: 808-813, April, 1968.

Serial No. NCI-4637
1. Biology Branch, OASDC,
Etiology
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Reports
July 1, 1967 through June 30, 1968

Project Title: Study of Soluble Antigen-Antibody Systems by C'la Fixation and Transfer

Previous Serial Number: Same

Principal Investigator: Tibor Borsos, Sc.D.

Other Investigators: Harvey Colten, M. D., Joel S. Spalter, M. D., and Herbert Rapp, Sc.D.

Cooperating Units: None

Man Years

Total:	1.5
Professional:	1.0
Other:	0.5

Project Description

Objectives:

To investigate the usefulness of carbodiimide and other coupling agents in the coupling of protein antigens to the erythrocytes of different species, to define the conditions suitable for this coupling, and to utilize these coupled antigens in immunological assays.

Methods Employed:

Egg albumin was coupled to human, sheep, and rabbit erythrocytes under various conditions of temperature, red cell and protein concentration, and incubation time. Optimal conditions for the production of stable coupled red cells were defined and these cells were used in several immunological assays including hemagglutination, direct passive lysis, and C'la fixation and transfer.

Major Findings:

Antibody dose-response curves were obtained in the C'la fixation and transfer test. Egg albumin coupled to rabbit cells with carbodiimide was used as antigen and the IgG and IgM fractions of rabbit anti-egg albumin serum were used as antibody. These dose-response curves were found to be similar to the dose-response curves obtained with the IgG and IgM fractions of rabbit antibodies specific for an antigen that is an integral part of the sheep cell surface.

Human red cells to which egg albumin was coupled were found readily susceptible to lysis by anti-egg albumin antibody and guinea pig complement.

Significance to Bio-medical Research and the Program of the Institute:

The coupling of antigens to red cells and the use of the C'la fixation and transfer test in conjunction with these coupled antigens provides a sensitive and quantifiable system for the detection of antibodies to soluble protein antigens. The system may provide a more sensitive and precise assay than is now available for rheumatoid factor and other abnormal serum proteins.

Soluble antigens of normal or neoplastic tissue might be coupled to red cells and antibodies to these antigens detected and measured with the C'la fixation and transfer test.

Proposed Course:

Tumor viruses and their antigens will be coupled by carbodiimide to red cells and the C'la fixation and transfer test will be used in the detection and characterization of antibodies to these viruses. Tumor and tissue specific protein antigens, when available, will also be studied with this system.

Serial No. NCI-4638
1. Biology Branch, OASDC, Etiology
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Biosynthesis and Ontogeny of Serum Complement

Previous Serial Number: Same

Principal Investigator: Harvey R. Colten, M. D.

Other Investigators: Tibor Borsos, Sc.D., James Gordon, M. D., and Herbert J. Rapp, Sc.D.

Cooperating Units:

Man Years

Total:	1.5
Professional:	1.25
Other:	0.25

Project Description

Objectives:

To identify the site or sites of synthesis of individual complement components by means of isolated organ and tissue culture, and to determine the effect of a number of chemicals (e.g. antibiotics, antimetabolites, carcinogens, etc.) on the synthesis of complement components; to identify and characterize serum complement components at various stages of embryonic and fetal development, and to identify the site or sites of complement synthesis in fetal tissues.

Methods Employed:

Guinea pig or human organs are isolated and maintained in vitro during the period of observation. Synthesis of the first component of complement (C'1) is detected by a technique based on the transfer of C'1 from tissue fragments to appropriate receptor cells. The number of molecules of C'1 synthesized can be calculated from the degree of hemolysis of the receptor cells. Methods for other components are under development.

De novo synthesis is confirmed by including ^{14}C-labelled amino acids in the incubation media and counting the ^{14}C-labelled complement component with standard counting equipment.

In addition localization of the cell types synthesizing complement *in vitro* are detected by a hemolytic assay performed in agarose gel.

Major Findings:

The first component of complement (C'1) is synthesized by isolated segments of guinea pigs' small intestine. This conclusion is based on the observation that incubation of segments of small intestine leads to an increase in C'1 production which can be inhibited by well known inhibitors of protein synthesis and that ^{14}C-labelled amino acids are incorporated into the C'1 molecule. We find that the columnar epithelial cell of the guinea pig small intestine is capable of *in vitro* C'1 synthesis. Under the conditions of the assay no other cell type was capable of synthesizing hemolytically active C'1.

Preliminary data obtained by injecting guinea pigs with ^{14}C-labelled amino acids indicate that the rate of synthesis of C'1 *in vivo* is quite rapid; maximal incorporation of label into the C'1 molecule occurs within three hours.

In related studies C'1 has been detected in sheep embryos early in gestation. Throughout the balance of fetal life serum C'1 content remains at about 20-30% of adult levels. Within two days of birth neonatal C'1 is within the adult range. This rapid rise in C'1 at birth is not due to ingestion of maternal colostrum, since colostrum has negligible levels of C'1.

Significance to Bio-medical Research and the Program of the Institute:

Synthesis of a complement component by small intestine is an example of "gut associated" control of immunologically active proteins. Because of the extreme sensitivity of the assay for the first component of complement, these studies provide some insight into the early events in the biosynthesis of proteins. In addition, the techniques used in this investigation provide a sensitive, rapid and easily performed assay for the effect of chemical and physical factors, including carcinogens, antibodies, steroids, ionizing radiation etc. on the synthesis of a protein.

Proposed Course:

The studies of complement synthesis and ontogeny of complement components in guinea pigs and sheep are now being extended to studies of human complement. Attempts to identify mechanisms controlling the synthesis of C'l may provide explanation for failures of host defense in a number of diseases, including patients with certain malignant diseases.

Publications

Colten, H. R., Gordon, J. M., Rapp, H. J., and Borsos, T.: Synthesis of the first component of guinea pig complement by columnar epithelial cells of the small intestine. J. Immun., 100: 788-798, 1968.

Serial No. NCI-4639
1. Biology Branch, OASDC, Etiology
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Mechanism of Complement Fixation and Action

Previous Serial Number: Same

Principal Investigator: Tibor Borsos, Sc.D.

Other Investigators: Harvey R. Colten, M. D., Joel Spalter, M. D.
and Herbert J. Rapp, Sc.D.

Cooperating Units:

Man Years
Total: 2.5
Professional: 1.0
Other: 1.5

Project Description

Objectives:

To apply rational complement fixation tests based on the fixation and transfer of the first component of complement to the analysis of antigen-antibody reactions, in particular in the search for cancer specific antigens.

Methods Employed:

The model for studying cytotoxic reactions mediated by antibody and complement consists of sheep erythrocytes, hemolytic antibody and guinea pig complement. Purification procedures for antibodies and the complement components include: preparative (large scale) gel filtration, ion exchange chromatography and preparative free electrophoresis. Other techniques used include precipitin and immunoelectrophoretic analysis, analytical and preparative ultracentrifugation and other immuno-and physicochemical methods.

Major Findings:

The ultimate goal of this work is the development of immunological tools that permit exploration of (1) immunological approaches to cancer diagnosis and (2) possible host immune defense mechanisms against cancer. The development of the C'la fixation and transfers test in this laboratory has permitted new approaches in investigating problems related to the above two areas of research. In studying the mechanism of complement fixation at cell surfaces we have discovered that temperature has a major effect on the efficiency of the fixation of the first component of complement by antibodies of the different immunoglobulin classes. In applying this test to the studies of the mechanism of complement fixation by antibodies of different immunoglobulin classes, we have discovered a heretofore unknown heterogeneity of antibody molecules belonging to the IgM class. We have found these molecules both in the rabbit and in the mouse. Our findings represent the first evidence that IgM molecules exist that are incapable of fixing complement.

In investigating the interaction of complement fixing sites at cell surfaces with the first component of complement, we have discovered that fixation of this component does not always result in its activation. Complexes made with 7S antibodies are particularly inefficient in their ability to activate the first component. This new finding may have far reaching consequences in our understanding of the action of complement in cytotoxic reactions for it indicates that under some conditions complement fixation may represent the removal of a component without it becoming capable of interaction with the other components.

Significance to Bio-medical Research and the Program of the Institute:

Complement fixation is one of the most widely used diagnostic tools. The development and successful application of a radically new and very sensitive C'F test, the C'la FT test, opens up new possibilities in determining antibody-antigen reactions on cell surfaces. The significance of this new test cannot be overemphasized since for the first time a test is available which gives molecular information about antibody-antigen reactions on cell surfaces. These studies on immune cytolysis have potential application to problems in the diagnosis and immunotherapy of cancer. Present evidence indicates that the essential features of the process of immune cytolysis are similar for a number of different kinds of target cells. Thus, information gained in studies on the mechanism of the lysis of erythrocytes by antibody and complement has served and will continue to serve as a guide in studies on immune lysis of cells, such as tumor cells, leukocytes, bacteria and protozoa.

Proposed Course:

This is a long range project, and we expect little change in the scope of the work during the next few years. The ultimate goals of these projects are the development of better diagnostic tools based on fixation of complement and the gaining of insight into the molecular events associated with the action of complement and antibodies. It is hoped that as a result of our program of inquiry into the basic problem of the interaction of antibodies, antigens and components of complement tools will be developed that are of practical significance in our search for cancer antigens.

Publications

Ishizaka, T., Ishizaka, K., Borsos, T., and Rapp, H.: C'1 fixation by human isoagglutinins: Fixation of C'1 by γ G and γ M but not by γ A antibody. J. Immun., 97: 716-726, 1966.

Rosse, W. F., Rapp, H. J., and Borsos, T.: Structural characteristics of hemolytic antibodies as determined by the effects of ionizing radiation. J. Immun., 98: 1190-1195, 1967.

Borsos, T., and Rapp, H. J.: Immune Hemolysis: A simplified method for the preparation of EAC'4 with guinea pig or with human complement. J. Immun., 99: 263-268, 1967.

Colten, H. R., Borsos, T., Rapp, H. J.: Efficiency of the first component of complement (C'1) in the hemolytic reaction. Science 158: 1590-1592, 1967.

Colten, H. R., Borsos, T., and Rapp, H. J.: Complement fixation by different classes of immunoglobulins: The effect of temperature. Protides of the Biological Fluids, 15: 471-477, 1967.

Rosse, W. F., Borsos, T., and Rapp, H. J.: Cold-reacting antibodies: The enhancement of antibody fixation by the first component of complement (C'la). J. Immun., 100: 259-265, 1968.

Plotz, P. H., Colten, H. R., and Talal, N.: Mouse macroglobulin antibody to sheep erythrocytes: A non-complement fixing type. J. Immun., 100: 752-755 , 1968.

Hoyer, L. W., Borsos, T., Rapp, H. J., and Vannier, W. E.: Heterogeneity of rabbit IgM antibody as detected by C'la fixation. J. Exp. Med., 127: 589-603, 1968.

Serial No. NCI-4651
1. Biology Branch, OASDC, Etiology
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Analysis of Murine Tumor Antigens and Antibodies by Complement Fixation and Immune Cytolysis

Previous Serial Number: None

Principal Investigator: Harvey R. Colten, M. D.

Other Investigators: Daniel Rubin, M. D., Tibor Borsos, Sc.D., and Herbert J. Rapp, Sc.D.

Cooperating Units:

Man Years

Total:	1.5
Professional:	1.25
Other:	0.25

Project Description

Objectives:

To apply the principles and methods of C' fixation and immune cytolysis, which have been developed in the hemolytic system, to the study of interaction between cytotoxic antibodies and tumor cell antigens. To study the nature and distribution of antigens on tumor cell membranes with methods which make both qualitative and quantitative distinctions possible.

Methods Employed:

Murine leukemias of both viral and non-viral origin have been maintained by serial transfer in ascites form in C57 Bl/6 mice. Antisera to these leukemias were prepared in mice and rabbits. Partially purified rabbit IgG and IgM antibodies were fractionated by gel filtration on sephadex G-200.

The cytotoxic reaction, detected by measuring electrolytic conductance, and the C'la fixation and transfer test were used to study quantitatively antigen-antibody reactions on the surface of tumor cells.

Major Findings:

Quantitative differences in the reactivity of syngenic mouse antisera directed against different, although related leukemias of viral origin (Friend, Moloney, and Rauscher), can be distinquished by means of the C'la fixation and transfer test. The results with individual antisera vary, however, due to the low titer of cytotoxic antibodies present.

Relatively high concentrations of leukemia cells are required to provide antigen excess for the titration of heterologous antibodies by C'la fixation and transfer. This finding, in addition to the kinetics of antibody sensitization, suggest that there are relatively few antigenic sites capable of binding heterologous antibody at the surface of murine leukemia cells.

Titrations of 7S and 19S rabbit antibodies against murine leukemias of both viral and non-viral origin by C'la fixation and transfer test show that one molecule of 19S rabbit antibody is sufficient to fix one C'la molecule whereas at least two molecules of 7S antibody are required for complement fixation on the surface of murine leukemia cells.

Comparison of quantitative measurements of rabbit 19S antibody to Rauscher leukemia cells by C'la fixation and transfer and immune cytolysis indicates that for IgM antibody molecules only one out of 3,000 complement fixing sites is cytolytic.

Significance to Bio-medical Research and the Program of the Institute:

The C'la fixation and transfer test provides a sensitive method for the study of the molecular interaction between cellular antigens and their respective antibodies. In this regard, it has been used to study antigens and antibodies induced by murine leukemia viruses. This technique makes it possible to characterize tumor antibodies and antigens on a molecular basis.

Preliminary results indicate that complement-fixation methods are capable of detecting viral-induced antigens and distinquishing among related tumor antigens induced by different viruses. In this manner, specific cytotoxic antibodies can be used to study the nature and distribution of tumor antigens in model systems which may lead to methods for detecting the appearance and development of human tumors. In addition these techniques can be used to characterize normal as well as neoplastic cells providing information regarding both quantitative and qualitative differences in antigenic structure.

Studies concerning the mechanisms of the cytotoxic reaction provide insight into the nature of one form of host defense. The development and application of precise quantitative techniques such as the C'la fixation and transfer test provide an approach to understanding this complex reaction on a molecular level.

Proposed Course:

Future efforts will be directed toward development and application of precise methods which are required to distinquish qualitative and quantitative differences among cellular antigens, specifically with respect to the detection of tumor-specific antigens. This will include continued testing of murine leukemias of the Friend-Rauscher-Moloney system by C' fixation and immune cytolysis to determine differences in the antigenic structure of related tumors induced by virus. Attempts will be made to obtain specific cytotoxic antibodies for differentiating a variety of murine leukemias of both viral and non-viral origin.

Serial No. NCI-4652
1. Biology Branch, OASDC, Etiology
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Antibodies to Complement Components

Previous Serial Number: None

Principal Investigator: Tibor Borsos, Sc.D.

Other Investigators: Harvey R. Colten, M. D., and Herbert J. Rapp, Sc.D.

Cooperating Units:

Man Years

Total:	1.5
Professional:	0.5
Other:	1.0

Project Description

Objectives:

To obtain highly purified antibodies that are capable of specifically interacting with guinea pig and human complement components. To develop methods for *in vivo* and *in vitro* assays for these antibodies.

Methods Employed:

Highly purified complement components in free form or bound to antibody antigen complexes are injected into appropriate recipient animals. Antisera obtained will be analyzed for anti-complement activity by inhibition assay, by precipitation reaction, and by agglutination reaction. Purification procedures of the immunoglobulins include preparative gel filtration, ion exchange chromatography and preparative electrophoresis. Specific antibodies will be isolated by elution from immune absorbants.

Major Findings:

We have been able to obtain rabbit anti-C'1 and anti-C'4 antibodies. These antibodies are capable of inhibiting the activity of these components and are capable of agglutinating cells that have these components on their surface. By the use of the anti-C'4 antibody we have been able to show that there may be as many as 4 different forms of this component on the cell surface; under usual conditions only one of them is capable of leading to hemolysis.

Significance to Bio-medical Research and the Program of the Institute:

Specific antibodies that are capable of interacting with complement components are useful tools in analyzing the reaction sequence leading to cell death. Such antibodies, furthermore, may yield information on the phylogenetic relationship of the complement components of different species and may be very useful in analyzing the ontogeny of complement components.

Proposed Course:

It is expected that during the next year this phase of the program will be devoted to large scale production of these antibodies. Once these antibodies are obtained in sufficient quantities they will be used to analyze the role of complement components in cytotoxic reactions.

CONTRACT NARRATIVE
BIOLOGY BRANCH, ETIOLOGY
Fiscal Year 1968

Cytogenetics and Cytology Section

TEMPLE UNIVERSITY, FELS RESEARCH INSTITUTE (PH 43-66-68)

Title: Effects of Organic Peroxides in Biological Systems

Contractor's Project Director: Daniel Swern, Ph.D.

Project Officer (NCI): Joseph A. DiPaolo, Ph.D.

Objectives: The purpose of this contract is to prepare, purify and/or isolate organic peroxides of various structural types having biological interest and significance for in house study. Commercially available peroxides will be examined for purity; stabilizing and other extraneous materials will be removed where feasible. Model compounds, structurally related to organic peroxides, will be prepared or purified from commercial sources, as will degradation products of organic peroxides.

In addition, the contractor agrees to synthesize new organic peroxides and lactones to meet the needs of the Cytogenetics and Cytology Section's research program.

Major Findings: The following is being carried out by the Section. Long term carcinogenesis experiments are underway using pure compounds belonging to a broad spectrum of peroxide structures. Distinct differences in maximum tolerated doses have been found. Because of toxicity problems the dose used for carcinogenic study requires alteration during the course of the experiments. Definitive experiments using a variety of strains of mice will require a minimum of 12 months to complete. On routine testing all peroxides appeared nonmutagenic or only slightly and inconsistently so. By setting up multiple identical platings (5-10) of each compound (one compound per plate) for each mutant and comparing the number of revertants obtained with spontaneous revertants on multiple blank platings, statistically significant results can be obtained. By this procedure several of the peroxides have been found to be mutagenic. Of those tested 5-butyl peracetate is the most active.

Significance to Bio-medical Research and the Program of the Institute: Even though hydroperoxides of simple structures have but transient existence in the living organism, the more complex organic peroxides are not affected by catalase or peroxidases. These peroxides, therefore, have a high potential for carcinogenic action independent of their abilities to cause the formation of epoxides which may be carcinogenic. The presence of these peroxides in photo-oxidized air and in heated fats to which the human population is exposed in a variety of ways, indicates the importance of the research in which these compounds will be used. It is self-evident that the continued exposure of individuals to organic peroxides, either environmentally or in foods, demands a thorough study of their biological effects.

If it is shown that organic peroxides have a deleterious effect in biological systems, steps can then be taken to destroy them or in some way neutralize their effect.

Proposed Course: Other samples of organic peroxides and derivatives will be prepared for examination of their biological effects. Not only will additional peroxides be prepared from classes originally agreed upon, but peroxides will be selected from other classes of interest to the program as it evolves. Some peroxides prepared during the first year of the contract will have to be remade for a continuing examination of their biological effects. Peroxides known to be present in or related structurally to autoxidized fats will be prepared, if time permits. The selection of peroxides will be made jointly by the Project Director and Project Officer of the NCI. An attempt will be made to provide enough samples of peroxides from selected classes so that meaningful and reliable correlations between structure and biological activity can be drawn.

Additional emphasis will be given by the contractor to the determination of the physical properties of the organic peroxides prepared, notably nuclear magnetic resonance, infrared and ultraviolet properties, with a view toward a characterization study of peroxide on a small scale. Preliminary studies of the kinetic decomposition of some of the peroxides may also be initiated to try to correlate decomposition rates (stability) with biological effects. Also selected gamma lactones will be prepared for study of the biological effects in comparison with organic peroxides.

Date Contract Initiated: December 1, 1965

Current Contract Level: December 1, 1967 - November 30, 1968: $24,450

INTERIOR, U. S. DEPARTMENT OF (NCI-FS-64-14)

Title: Abnormal Lipid Metabolism in Salmonoid Fishes. Aflatoxin and Other Feed Components as Liver Carcinogens for Trout.

Contractor's Project Director: Dr. John E. Halver

Project Officer(s) (NCI): Dr. Elizabeth K. Weisburger

Objectives: To identify hepatoma inducing components of commercial trout diets; to determine the minimal exposure to aflatoxin which may lead to hepatoma in trout; to describe and report detailed histopathology of the effects of carcinogens on trout liver.

Major Findings: During the past year it was determined that coho salmon did not develop hepatomas when fed relatively high levels of aflatoxin B_1 in the diet for 20 months. For trout it was found that dietary levels of aflatoxin less than 0.05 ppb would probably not produce hepatoma over a 20 month period. A minimum effective dose was about 0.1 ppb. Very young trout were more sensitive to aflatoxin B_1, analogous to results in other species with other carcinogens. Several analogs of aflatoxin, made available from the synthetic organic work of Dr. Büchi at MIT, had very low tumorigenic activity. Histopathological evaluation of the material from 5 hepatoma induction experiments continues.

Significance to Bio-medical Research and the Program of the Institute: This program is related to other studies sponsored by the NCI on carcinogenic toxins in foodstuffs (MIT - PH43-62-468; Temple - PH43-65-1029; City of Hope - PH43-65-1036). The results have a bearing on problems of food processing and storage for man and animals. The data obtained under this contract have provided an explanation for the 1960 epidemic of hepatoma in hatchery-raised trout in the United States and abroad.

Proposed Course: The remaining slides are to be examined and the evaluation of the data is to be completed. The contract is due to expire this year.

Date Contract Initiated: January 1, 1961

Current Annual Level: FY 1968: $12,000

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (PH43-62-468)

Title: Toxicity and Carcinogenicity Associated with Fungal Growth on Foodstuffs

Contractor's Project Director: Drs. Gerald Wogan and George Büchi

Project Officer(s) (NCI): Dr. Elizabeth K. Weisburger

Objectives: To determine the agents causing alteration of foodstuffs or production of toxic and carcinogenic metabolites; to study the mechanisms by which such materials are produced; to determine the effects of these materials on cellular processes; and to have an exact knowledge of naturally occurring carcinogens with a view to preventing such conditions.

Major Findings: The investigations by the organic chemical group (Dr. Büchi) indicate that the biosynthetic pathway of aflatoxin is different from other biosynthetic schemes thus far proposed. A tentative structure for rubratoxin has been developed. The synthesis of metabolites of aflatoxin is being pursued.

The biochemical studies (Dr. Wogan) of effects of aflatoxin on enzymic processes and nuclear function are continuing. The quantitative separation of unconjugated and conjugated aflatoxin metabolites in rat urine has been achieved. Possible cocarcinogenic effects of aflatoxin and rubratoxin are being investigated.

The MIT group continues to supply aflatoxin, both as a standard and for various experimental purposes, to many investigators all over the world.

Significance to Bio-medical Research and the Program of the Institute: Adulteration or alteration of foodstuffs by microbiological agents may be of considerable importance in view of epidemiological data showing high cancer incidence at various anatomical sites in certain areas of the world. The high liver cancer incidence in Africa or Southeast Asia may possibly be a consequence of such microbiological agents. This project is related to other contracts on carcinogenic natural products supported by NCI.

Proposed Course: These experiments on all aspects of mycotoxin research constitute an important long-term program, with a superb team of investigators. Determination of the biosynthetic pathway of aflatoxin will eventually lead to methods for blocking the growth of the toxin. Study of the metabolic pathways of aflatoxin in various species may lead to a screening procedure for human population surveys on aflatoxin ingestion.

Date Contract Initiated: June 25, 1962.

Current Annual Level: November 1, 1967 - October 31, 1968: $153,437

CONTRACT NARRATIVES
Biology Branch, Etiology
Fiscal Year 1968

Bioassay Section

BIONETICS RESEARCH LABORATORIES, INC. (PH43-67-735)

Title: Carcinogenesis Studies Including the Monitoring of Pesticides

Contractor's Project Director: Dr. J. R. M. Innes

Project Officer (NCI): Dr. Richard R. Bates

Objectives: To evaluate the carcinogenicity, teratogenicity and chronic toxicity of pesticides and other environmental agents; and to develop analytical methods for detecting these and related compounds and to study their degradation and metabolism.

Major Findings: The Chemistry program has resulted in an extensive bibliography of papers on techniques for the identification of pesticides and the synthesis of their chemical analogues. This portion of the project has been terminated. Autopsies have been completed on all animals which were given the 129 different pesticides and environmental agents to test their chronic toxicity and carcinogenicity. It is anticipated that histologic processing of tissues will be completed by June 1968. Reading of slides will be finished shortly thereafter. It is apparent now that the great majority of the chemicals tested are not carcinogenic. A small number appear to be so, but statistical evaluation of the data has just begun.

Selected compounds representing various chemical classes of pesticides and environmental agents are being tested for teratogenicity. Most have proven non-teratogenic when large doses are injected into pregnant mice. The few compounds which did produce anomalies are being re-studied by the oral route of administration.

Significance to Bio-Medical Research and the Program of the Institute: There is widespread human exposure to these compounds as a result of their extensive use. Thus, it is important to develop techniques to detect their presence and to determine whether they are a health hazard to mammals.

Proposed Course: The contract is scheduled to end on August 25, 1968

Date Contract Initiated: November 26, 1963 (formerly PH43-64-57)

Current Annual Level: February 25, 1968 - August 25, 1968: $140,000

CONTRACT NARRATIVES
BIOLOGY BRANCH, ETIOLOGY
Fiscal Year 1968

Immunology Section

CORDIS CORPORATION (PH43-66-936)

Title: Purification of Complement Components from Human Serum, and Standardization of Reagents used in their Assay.

Contractor's Project Director: S. P. Halbert, M. D.

Project Officer (NCI): Tibor Borsos, Sc.D.

Objectives: To obtain highly purified and active complement components of human origin. To furnish sufficient amounts of guinea pig complement components to satisfy in-house activities of the Immunology Section, Office of the Chief, Biology Branch, NCI and other investigators at NIH in need of such reagents.

Methods Employed: Physical chemical separation of these serum proteins is accomplished by: low ionic strength precipitation at pH 7.5 and 5.5, salting out with high concentrations of ammonium sulfate, DEAE- and CM- cellulose column chromatography, and gel filtration with Sephadex. These components will be quality controlled at Cordis for hemolytic activity and for purity.

Major Findings: This is a contract which calls for the development of large scale techniques based on existing small scale production methods. To date, contractor has supplied two shipments of all nine components of human complement and one shipment of the first and second components of guinea pig complement. Tests in our laboratory indicated that most of these components were satisfactory. Difficulties were encountered with the stability of the first and second components of complement and unsatisfactory separation of the sixth, seventh and eighth components. These problems are now under investigation.

Significance to NCI Program & Bio-medical Research: Complement participates in cytotoxic reactions in conjunction with specific antibodies. Complement fixation furnishes a sensitive and precise tool for the measurement of antibody-antigen interactions. Both the cytotoxic and complement fixation reactions are extensively used in immuno-chemical characterization of tumor antigens.

In addition, antibody and complement play an important role in body defenses against foreign antigens; it is hoped that the availability of purified complement components will aid in the identification of immune mechanisms responsible for tumor rejection.

Proposed Course: To continue the production of complement components.

Date Contract Initiated: June 21, 1967

Current Annual Level: June 21, 1967 to June 20, 1968, $99,000

CONTRACT NARRATIVE
BIOLOGY BRANCH, ETIOLOGY
Fiscal Year 1968

Office of the Chief

UNIVERSITY OF CALIFORNIA SCHOOL OF MEDICINE, SAN FRANCISCO (PH43-64-42)

Title: Response of Mammalian Respiratory Epithelium Grown in Organ Culture to Carcinogenic and Non-carcinogenic Hydrocarbons

Contractor's Project Director: T. Timothy Crocker, M.D.

Project Officer (NCI): Doris P. Courington, M.D.

Objectives: To investigate the response of primate (human and monkey), dog, and rodent (hamster, rat) respiratory epithelium to environmental polycyclic hydrocarbons; to develop an in vitro bioassay system for studying the effects of diverse potential and known carcinogens; to obtain data pertinent to the pathogenesis of lung cancer in man.

Major Findings: The concentration of effort during this final year has been to extend the system of organ culture of the tracheobronchial tree to include specimens from dogs and from primates - the Rhesus, Cynomolgus and Galagos and human fetal tissues. Previously reviewed techniques of administration of known carcinogens and non-carcinogens were used and histological, cytological and autoradiographic evaluations of the response of the epithelium were made and compared with data available on the hamster cultures. In the hamster, the first and most marked effect was on the connective elements, in contrast to the primates where the most striking change was the production of basal hyperplasia, and proliferation of poorly differentiated cells at the time the connective tissue showed morphologic integrity and labeling. Further evaluation of kinetics of DNA metabolism is still under way.

Significance to Bio-medical Research and the Program of the Institute: The similarity of the responses seen in the primate organ culture to those reported elsewhere in the intact animal is encouraging, suggesting that at least potentially carcinogenic compounds might be pinpointed with this screening technique prior to extensive testing on large animal colonies.

Proposed Course: Contract is scheduled to be completed October 31, 1968.

Date Contract Initiated: January 23, 1964

Current Annual Level: Funding through February 31, 1968: $43,192
Contract was extended without additional funds until October 31, 1968

UNIVERSITY OF PITTSBURGH (PH43-64-504)

Title: Biochemical and Morphological Components of Hepatic Carcinogenesis

Contractor's Project Director: Emanuel Farber, M.D., Ph.D.

Project Officer (NCI): Doris P. Courington, M.D.

Objectives: To feed known or suspected carcinogens of diverse chemical structure with a basic diet of known composition and study the animals for the production of hyperplastic nodules and/or tumors; to study the hyperplastic nodule as the precursor of neoplasms and to pinpoint biochemical characteristics which may serve as precise end-points for an assay system for hepatocarcinogens as distinguished from hepatotoxins.

Major Findings: Three compounds, aflatoxin B-1, thiuram and griseofulvin were tested for nodule and/or tumor production, with positive results for nodules and tumors in the case of aflatoxin B-1 only. In addition to primary hepatomas, renal cell carcinoma was present in a large number of those on the highest dose level. Renal tumors have not previously been reported with this compound. The binding of the carcinogen to abnormal glycogen deposits was noted in the case of one of the prototype compounds, acetylaminofluorene, long after the feeding of the compound had ceased. The binding of the compound may explain the resistance of the glycogen to glycolytic enzymes. It might also provide a prolonged exposure of a cell to a carcinogen.

Significance to Bio-medical Research and the Program of the Institute: Certain distinct biochemical differences have been found in some hyperplastic nodules as compared to histologically normal surrounding liver tissue. If these alterations prove to be common to all hyperplastic nodules produced by carcinogens, they may serve as sensitive end-points for an assay system.

Proposed Course: The nodules produced by aflatoxin will be studied in detail with regard to glycogen metabolism, binding of the carcinogen and effect on DNA synthesis and cell mitosis. The use of Fischer strain which is inbred and will allow transplantation of nodules will allow further testing of the relationship of the hyperplastic nodules to tumor production.

Date Contract Initiated: January 20, 1964

Current Annual Level: January 31, 1968 - January 31, 1969: $31,000

UNIVERSITY OF CALIFORNIA, DAVIS (PH43-64-579)

Title: Mouse Mammary Nodulogenesis and Tumorigenesis Induced by Chemical Carcinogens

Contractor's Project Director: Leslie J. Faulkin, Ph.D.

Project Officer (NCI): Doris P. Courington, M.D.

Objectives: To develop and standardize a bioassay test system in carcinogenesis using the mouse mammary gland; to determine the influence of different chemical agents including the known chemical carcinogens and related non-carcinogenic derivatives in this system; to test the influence of chemical and viral agents, given simultaneously on carcinogenesis in the same system.

Major Findings: Previous *in vivo* and transplantation studies with mammary tumor virus by the contractor has indicated that the hyperplastic nodule produced with the virus is a preneoplastic lesion. The contract was developed in order to test the production of similar lesions by chemical carcinogens and to establish if these lesions have a comparable tumor production rate. In the initial phase, the carcinogens were administered by gavage and simultaneous hormonal stimulation accomplished by pellet implantation of estrogens and adrenocorticoid hormones or by pituitary implants. In the past year *in vitro* organ culture has been used to further test the *in vivo* results. Transplantation of the whole mammary fat pad from mice and rats previously receiving hormonal stimulation has been accomplished, with subsequent development of fin ducts and alveolar proliferation and secretion. Such proliferating cultures are treated with carcinogens and re-implanted for prolonged study. The project has been complicated by the discovery of a virus particle (in the BALB/c strain) at the electron microscope level of a particle closely resembling mammary tumor virus. Biological activity is not yet established. Testing has been extended to include 20 additional compounds, including nitrofuran derivatives, aromatic amines and polycyclic aromatic hydrocarbons. In addition to the above mentioned hormonal stimulation, contraceptive pills are being used in one group.

Significance to Bio-medical Research and the Program of the Institute: Because of the ease of re-implantation or translocation of the mammary fat pad and gland this test system holds considerable promise as an assay useful in evaluating carcinogens. In addition, as a hormonal target organ with a characteristic cellular response, it offers a more precise mechanism for investigating the role of hormones in tumorigenesis. The extension of the testing to the rat may help solve the problem of co-carcinogenesis posed by the presence of a mammary tumor virus-like particle in the BALB/c strain of mice.

Proposed Course: It is expected that the compounds now under test will give us a clear cut idea of the validity of the test system and that the contract will expire at the end of the current experiments.

Date Contract Initiated: June 24, 1964

Current Annual Level: January 1, 1968 - December 31, 1968: $50,564

SUMMARY REPORT
CHEMISTRY BRANCH
July 1, 1967 - June 30, 1968

The program goal of the Chemistry Branch is to elucidate the fundamental mechanisms of carcinogenesis; to investigate the sequence of molecular events by which carcinogenic agents, chemical, physical, and viral, induce neoplasia, and the exogenous and endogenous factors which modify the carcinogenic process; to investigate the chemical or physical interaction of carcinogenic agents with living organisms at the host, tissue, cellular and molecular levels, and to clarify the functional consequences of these interactions.

Carcinogenesis, reflected by a changing and heritable phenotype, seems to involve alterations in the pattern of genetic expression. The Chemistry Branch studies the biochemical nature of factors controlling gene expression and messenger-RNA translation as well as the chemical nature of the genetic factors characterizing susceptibility to carcinogenesis.

Carcinogens have been found to alter gene expression as shown by their activity in the induction of specific microsomal enzymes. The nature of this mechanism has been difficult to study in the whole organism. Methods have been developed to study the induction of an enzyme system metabolizing carcinogens in the controlled environment of tissue culture. Thus, considerable methodological advances have been made for studying the intimate biochemical events by which carcinogens alter genetic expression and induce specific enzymes.

A sensitive assay has been developed for the detection of this aryl hydroxylase enzyme and the properties of the enzyme and induction system have been established. Enzyme level is increased up to 40-fold when the cells are grown in the presence of polycyclic hydrocarbons. The enzyme system studied is a key one in the detoxification of a variety of carcinogenic agents, drugs and atmospheric pollutants, and its level in the organism is significantly related to the pharmacology and toxicology of these agents. Knowledge of the mechanism controlling its level is of vital importance to understanding the organism's response to environmental agents.

A major advance in RNA methodology has been developed, which will considerably further investigations on the mechanism of carcinogenesis, hormone action, and differentiation. The application of these methods to other areas of biochemical research will be extensive.

New methods involving polyacrylamide gel electrophoresis of RNA have been developed for the analysis and separation of RNAs ranging in size from 10^4 to 10^8 daltons. The method depends on the use of acrylamide gels strengthened with agarose for analysis of the larger RNAs. The resolving power of the method permits individual characterization of RNAs in mixtures containing multiple species of RNA, without prior purification of each species; RNA molecules which differed in molecular weight by only a few per cent could be clearly distinguished, and the molecular weight of each estimated. This unusual and new method for determination of RNA molecular weight is based on the observation that the mobility and the logarithm of the molecular weight are inversely related and this relationship is approximately linear. The molecular weights estimated by this technique are highly reproducible in gels of various composition, and are the best means of identification of species resolved by gel electrophoresis. By this means, liver 18S RNA is identified as a doublet of RNAs of 0.66 and 0.62×10^6 daltons and the analogous 16S of E. coli as a doublet of 0.58 and 0.54×10^6 daltons, while liver 5S RNA has a molecular weight of 38,000 daltons.

The mobility of the several species of RNA is altered differently depending on the magnesium composition of the buffer, and this behavior may provide an additional parameter for the characterization of these RNAs. Thus, the mobility of 30S RNAs are specifically slowed more than 18S RNA.

This new method has also been applied to the study of the effect of various carcinogenic and non-carcinogenic analogs on the transcription of genetic information into RNA and to studies on the mechanism of hormone action.

Previous studies indicated a correlation between the carcinogenicity of various aminoazo dyes in rat liver and the inhibition of RNA synthesis. This was further studied with several carcinogenic nitrosamine derivatives. No correlation was observed between their carcinogenicity and their effect on RNA synthesis. Thus, the results suggest a difference in the modes of action between the carcinogenic aminoazo dyes and the nitrosamines. The effect of carcinogenic agents on the pattern of RNA synthesis was studied by polyacrylamide gel electrophoresis. The RNA patterns from carcinogen-treated rats was similar to that of the control rats, indicating that the inhibition of RNA synthesis by the carcinogen is not directed to a single species of RNA but rather that most species of nuclear RNA are inhibited to about the same extent.

The enzyme inducer and carcinogen 3-methylcholanthrene stimulates RNA synthesis in isolated rat liver nuclei; the pattern of RNA synthesis during MC treatment was examined by polyacrylamide gel electrophoresis. The overall pattern of isotope incorporation was greatest at three hours after the methylcholanthrene was administered. Methylcholanthrene induced unique changes in the pattern of the nuclear 5-10 S RNA. These species of RNA may have a regulatory function.

Both actinomycin and aflatoxin, a potent liver carcinogen, cause a marked inhibition of RNA synthesis with the rapid loss of high molecular weight RNA species from the nucleus. After a high degree of blockage of RNA synthesis with actinomycin, the rapidly labelled high molecular weight RNA appears to be converted to lower molecular weight nuclear RNA and cytoplasmic RNA. This conversion is not significantly inhibited by actinomycin.

This new technique of RNA separation has been applied to study the mechanism of steroid hormone action in immature chick oviduct and specifically to study the effect of the steroids on nuclear RNA and cytoplasmic RNA metabolism. The major finding has been a large increase in the amount of transfer RNA in the nucleus of oviduct cells from chicks receiving estrogen stimulation, and a smaller increase in the amount of transfer RNA present in the cytoplasm. The newly synthesized nuclear RNA of the unstimulated oviducts seems to be more rapidly degraded and/or converted to lower molecular weight species than is the newly synthesized RNA from the stimulated oviducts.

In another study utilizing the new method for RNA separation, the mechanism of aldosterone action in rat kidney has been studied. The pattern of RNA present in kidney cell nuclei and cytoplasm was unchanged after treatment with aldosterone.

A major aspect of regulation of growth involves the control of messenger RNA degradation. A major effort of the branch concerns the enzymes of tumor cells responsible for the *in situ* destruction of nuclear RNA. An exoribonuclease from Ehrlich ascites tumor cells has been found to degrade messenger-type RNA from the 3'-OH end to produce 5'-mononucleotides. The enzyme remains complexed with an individual polynucleotide molecule until it has been totally degraded. A new molecular model for this type of continuous degradation has been developed and tested in the laboratory. It is believed that the enzyme has six principal cationic sites which keep the polynucleotide bound during the process of degradation. Three new compounds, namely 1) thymidine-3',5'-difluorophosphate, and 3) thymidine-3'-*p*-bromo-acetanildophosphate inhibit over 50% of the Ehrlich exoribonuclease *in vitro*. These compounds were synthesized for NCI by Ash Stevens, Inc., Detroit, Michigan, under Contract Number PH 43-66-929.

Other studies on the control of genetic expression have involved the mechanism of regulation of the translation of RNA into protein. One requisite of this type study is the isolation of the functional unit of protein synthesis. Thus, methods for the isolation of ribosomes have been developed. Bacterial polysomes are resolved on acrylamide gels with discrete bands up to octamer size. The bands correspond to polysomes obtained by sucrose gradient fractionation. When polysomes in a gel are electrophoresed a second direction after detergent treatment, 16S and 23S RNA bands are observed for each polysome band. Ribosomes which migrate in a gel as 70S monosomes in 10^{-2} M Mg, dissociate at lower magnesium concentrations and are resolved into two types of 30S subunits and a 50S subunit in the gel. The two 30S subunits have identical 16S RNA and the 50S subunit has a 23S RNA.

In a parallel study these methods have been used to separate the proteins of endoplasmic reticulum of rat liver. These membrane components are believed to be important in the control of protein synthesis and are considerably altered during carcinogenesis.

The electrophoretic separation of these proteins revealed approximately 15 bands. Further studies utilizing solubilization and gel filtration techniques suggest that these proteins can be associated *in vitro*. These studies may contribute to an understanding of normal microsomal structure. Since microsomal enzymes are important in the structural alteration of drugs and may contribute to the detoxification of known or unknown carcinogens, such an analysis would facilitate the study of carcinogenic alteration.

A major effort of the laboratory is the study of the biochemistry of oncogenic transformation by Rous Sarcoma Virus. The highly controversial Warburg hypothesis of a defect in energy metabolism in tumor cells has been examined in RSV transformed cells.

No characteristic difference in lactic acid production was observed between normal and RSV transformed cultures in a state of optimal growth. Under conditions limiting cell multiplication, glycolysis declined commensurate with growth rate in both control and transformed cultures. However, cultures infected with Rous Sarcoma Virus were less inhibited than the uninfected normal cells. Thus, transformed cells showed a differential increase in rates of lactate production and protein accretion under conditions of contact inhibition, serum depletion, thymidine block and culture in liquid suspension medium. These differences were generally evident within two days of viral infection. The studies indicate that transformation of chick embryo cells by Rous Sarcoma Virus enhanced their capacity for growth and that the augmented glycolytic rates may be due to the expression of a greater growth potential.

A transient requirement for DNA synthesis in the growth cycle of avian tumor viruses, and in transformation by Rous sarcoma virus, was established previously.

Experiments with murine sarcoma virus, an RNA tumor virus of mammals, have revealed a similar requirement for DNA synthesis. Other biological features of infection by Rous sarcoma virus which reflect the requirement for DNA synthesis were noted also with murine sarcoma virus.

These and other experiments suggested that a new viral DNA was synthesized in cells infected with RNA tumor viruses. Utilizing nucleic acid hybridization techniques, the identification of a new DNA was attempted by comparing cellular DNAs (normal and infected) in their capacity to bind viral RNA. The binding of viral RNA was found to be greater to DNA from infected cells than to DNA from noninfected cells. This result was consistent with the idea that a new virus-specific DNA is synthesized in infected cells.

Other studies involving the characterization of the internal components of avian tumor viruses have shown that the outer lipid-containing envelope of Rous sarcoma virus can be removed from a central nucleocapsid with mild detergent. The internal nucleocapsid contains the viral RNA, is spherical and has a dimension of the volume enclosed by the "intermediate membrane" of whole virus. Whereas intact virus has a buoyant density of 1.16 gm/cc in sucrose, K tartrate, or Cs_2SO_4 solution, the nucleocapsid bands at 1.26 gm/cc in these solutions.

The RNA from intact virions has been extracted and characterized by sedimentation in sucrose gradients and electrophoretic migration in polyacrylamide-agarose gels. In addition to the large (10^7 mol. wt.) RNA described by others, RNA with characteristics of cellular ribosomal RNA was also found.

Other studies have investigated the characteristics of murine cells transformed by murine sarcoma virus. A tumor induced in rats by murine sarcoma virus - murine leukemia virus mixture - was grown in cell culture. Although biological techniques failed to detect virus, presence of virus-like particles in cell culture fluids was demonstrated by electron microscopy and confirmed by radioactive labelling and purification of the particles. The addition of leukemia virus to the cultures resulted in the production of infectious sarcoma virus. These studies suggest that murine sarcoma virus may be produced which has a host range different from that of a related leukemia virus, but by mixed infection the host range of the sarcoma virus can be expanded.

In another study the T antigen from Simian Virus 40-induced tumors has been purified and partially characterized. Although not isolated in pure form the T antigen has thus far been purified several hundred-fold over its concentration in the intact tumor cell. Results obtained by immunodiffusion and immunoelectrophoresis suggest that T antigen is a single protein that theoretically could be separated from all other types of cellular proteins.

Studies on the mechanism of differentiation and A C T H action in adrenal cells has shown an increase in the amount of synthesis of an RNA fraction sedimenting as 4S. This RNA type has been implicated in the control of protein synthesis, and preliminary studies suggest that glutamine transfer RNA is changed during ACTH action. Also, the chromatographic properties of glutamine synthetase appear altered.

A new study is being developed which may clarify the relationship between various transfer RNA species and the control of protein synthesis and differentiation. Methodology has been set up on the separation and characterization of the activities of various transfer RNAs from different tissues.

Electron microscopy and radioautography have been used to study the mechanism of DNA synthesis in mammalian cells, which may be a central problem of carcinogenesis. Experiments suggest that DNA replication in mammalian cells is initiated at a nuclear membrane. The data suggest that there are considerably more than two replication sites in a single chromosome and that nucleolar RNA synthesis depends on DNA synthesis. Others have shown a specific template activity of nucleolar DNA for nucleolar or ribosomal RNA synthesis. This may relate to the transient requirements of DNA synthesis for viral RNA replication in Rous sarcoma and certain murine leukemia infections. These studies have established a base for the integration of the metabolic and morphologic studies of DNA replication in mammalian cells.

In other studies there has been an extension of the findings relating haptoglobin type to differential susceptibility to leukemia and other diseases.

Two hundred thirty-four normal control specimens obtained from the Clinical Center Blood Bank were added to our control series to make a total of 426 normal patients studied. The second set was nearly identical to that observed previously. The total numbers are as follows: type 1-1, 56; type 2-1, 189; type 2-2, 181. Four hundred five female patients with cancer of the breast were distributed as follows: type 1-1, 57; type 2-1, 196; type 2-2, 142. Detailed statistical analysis indicates that these two populations are statistically different, but we are not able to rule out the possibility of some variable other than cancer of the breast. Haptoglobin subtyping analysis which had been performed on the serums from women with cancer of the breast were compared with similar results obtained from the normal control studies made this past year. Both series show substantially greater frequencies of the "F" gene than we have been led to expect from published studies of others.

A study is in progress on the use of disc electrophoresis to detect and to identify hemoglobin variants in red cell hemolysates. The analysis of these variants is important for diagnosis in certain disease states, and also in studies of the biochemical genetics of hemoglobin synthesis.

A study is in progress on the nature of the unusual reaction between hemoglobin and haptoglobins. The reaction is irreversible and proceeds stepwise through the formation of intermediate complexes to the complete complexes of the haptoglobin-hemoglobin combinations. Several of the multiple bands of the haptoglobins 2-1 and 2-2 types were individually isolated for this study, by the careful excision of the unstained protein bands from the disc electrophoretic gel columns.

Serial No. NCI-4716
1. Chemistry Branch, OASDC, Etiology
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Metabolic Requirements for Infection by RNA-Containing Tumorigenic Viruses

Previous Serial Number: 4716, 4725

Principal Investigators: John P. Bader, Yozo Nakata, Harriette Phelps

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 2.8
Professional: 2.3
Other: 0.5

Project Description

Objectives:

To determine the intracellular biochemical processes involved in the growth of RNA-containing tumor viruses and in malignant transformation by these viruses.

Methods Employed:

Cell culture, radioactive tracer techniques, extraction of cellular macromolecules, virus assay by focus formation, X-irradiation.

Major Findings:

A transient requirement for DNA synthesis in the growth cycle of avian tumor viruses, and in transformation by Rous sarcoma virus, was established in previous years.

The required DNA was synthesized during the early stages of infection. A single cellular cycle of DNA synthesis was sufficient for virus growth or for transformation. Similar experiments with murine sarcoma virus, an RNA tumor virus of mammals, have revealed a similar requirement for DNA synthesis. Other biological features of infection by Rous sarcoma virus which reflect the requirement for DNA synthesis were noted also with murine sarcoma virus.

These and other experiments suggested that a new viral DNA was synthesized in cells infected with RNA tumor viruses. Identification of a new DNA was attempted by comparing cellular DNAs (normal and infected) in their capacity to bind viral RNA, utilizing nucleic acid hybridization techniques. The binding of viral RNA was found to be greater to DNA from infected cells than to DNA from noninfected cells. This result was consistent with the synthesis of a new virus-specific DNA in infected cells.

Significance to Biomedical Research and the Program of the Institute:

The requirement for DNA synthesis seems to be a general characteristic of the RNA -containing tumor viruses. Such information is vital to an understanding of viral tumorigenesis, and the eventual control of infection by RNA tumor viruses may depend upon such studies.

Proposed Course:

To further characterize the DNA required for infection, and the mechanism of its synthesis.

Honors and Awards

Invited to Lecture:	Symposium "Comparative Leukemia Research," International Leukemia Conference, Paris, France, July 1967.
	University of Maryland Medical School, Baltimore, Maryland, February 1968.

Serial No. NCI 4726
1. Chemistry Branch, OASDC
Etiology
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Glycolysis in Chick Embryo Cultures Infected with Rous Sarcoma Virus

Previous Serial Number: NCI 4726

Principal Investigators: Theodore L. Steck, Seymour Kaufman and John P. Bader

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1.0
Professional: 0.8
Other: 0.2

Project Description

Objectives:

To determine if characteristic differences in glucose metabolism exist between tumor and non-tumor cells.

Methods Employed:

Cell culture, cell growth studies; assay of glucose, lactic acid, lactic dehydrogenase (LDH) and related parameters; radioactive labeling.

Major Findings:

Glycolysis has been evaluated in situ in chick embryo cell cultures transformed with Rous sarcoma virus. No characteristic difference in lactic acid production was observed between normal and transformed cultures in a state of optimal growth. Under certain conditions chosen to limit cell multiplication, glycolysis declined commensurate with growth rate in both control and transformed cultures. However, cultures infected with Rous sarcoma virus were less inhibited in this respect than their uninfected counterparts. Thus, transformed cells showed a differential increase (i.e., less inhibition) in rates of lactate production and protein accretion under conditions of contact inhibition, serum depletion, thymidine block and culture in liquid suspension medium. These differences were generally evident within two days of viral infection. It was concluded that transformation of chick embryo cells by Rous sarcoma virus enhanced their capacity for growth and that augmented glycolytic rates appeared only as this differential growth potential was expressed.

Significance to Bio-Medical Research and the Program of the Institute:

The observation of enhanced glycolysis in tumors is a major theme in cancer research. The mechanism of apparent glycolytic alterations has been widely debated. The present study sought to employ well controlled experimental conditions to examine such metabolic changes. Our results indicated no constitutive alteration in glycolysis in transformed cells. Rather, we observed a differentially enhanced growth potential in Rous sarcoma cells, which was accompanied by a commensurate rise in glycolysis. Attention should be thus focused on the more proximal events leading to these alterations in the growth state.

Proposed Course:

Since it appears that changes in glycolytic activity in chick embryo cells transformed by Rous sarcoma virus do not represent irreversible or proximal events, we shall suspend further studies in this area. The conditional alterations in glycolytic rates may be assayed in future studies as one additional metabolic parameter accompanying viral transformation.

Serial No. NCI-4735
1. Chemistry Branch, OASDC
   Etiology
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: An Analysis of Cells Transformed by Murine Sarcoma Virus

Previous Serial Number: 4727

Principal Investigators: John P. Bader and Artrice F. Valentine

Other Investigators: None

Cooperating Units: Viral Biology Branch

Man Years:
Total: 0.8
Professional: 0.8
Other: None

Project Description

Objectives:

To investigate the character of murine cells transformed by murine sarcoma virus and to correlate ultrastructural characteristics with biological activities.

Methods Employed:

Cell culture, virus assays by quantitative transformation, radioactive labelling, density gradient centrifugation, electron microscopy.

Major Findings:

A tumor induced in rats by murine sarcoma virus, murine leukemia virus mixture, was grown in cell culture. No infectious sarcoma or leukemia virus could be demonstrated in such cultures by biological techniques which readily detected the original infecting virus. However, the presence of virus particles in cell culture fluids was demonstrated by electron microscopy and confirmed by radioactive labelling and purification of the particles. Also, the addition of leukemia virus to such cultures resulted in the production of infectious sarcoma virus. These studies suggest that, as in the case of avian tumor viruses, murine sarcoma virus may be produced which has a host range different from that of a related leukemia virus, but by mixed infection the host range of the sarcoma virus can be expanded.

Significance to Bio-Medical Research and the Program of the Institute:

These studies increase our understanding of the mechanism of reproduction of RNA-containing tumor viruses, particularly the early and late stages involving acquisition of the viral envelope and the specificity of the envelope for reinfection of cells.

Proposed Course:

Similar studies analysing several selected lines of rat cells transformed by murine sarcoma virus.

Publications

1. Bader, J. P. and Valentine, A. F., Biochemical Studies of Cells Infected with RNA Tumor Viruses. Symposium "Comparative Leukemia Research" (in press).

2. Valentine, A. F. and Bader, J. P., Production of Virus by Cells Infected with RNA-Containing Tumor Viruses, J. Virology (in press).

Serial No. NCI-4736
1. Chemistry Branch, OASDC
   Etiology
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Analysis of the Nucleocapsid and Nucleic Acid of Avian Tumor Viruses

Previous Serial Number: None

Principal Investigators: John P. Bader, Theodore L. Steck, Nancy R. Brown

Other Investigators: Artrice F. Valentine

Cooperating Units: Electron Microscopy Section, Viral Biology Branch, NCI

Man Years:

Total:	1.8
Professional:	1.3
Other:	0.5

Project Description

Objectives:

To characterize the internal components of avian tumor viruses and to relate these to growth of virus and malignant transformation of cells by these viruses.

Methods Employed:

Cell culture, radioactive tracer techniques, density-equilibrium and sedimentation-rate centrifugation, preparation and purification of nucleic acids, selective molecular extractions, polyacrilamide-gel electrophoresis, electron microscopy.

Major Findings:

The outer lipid-containing envelope of Rous sarcoma virus can be stripped from a central nucleocapsid using mild detergent. The internal nucleocapsid is spherical with dimensions of the volume enclosed by the "intermediate membrane" of whole virus. Whereas intact virus has a buoyant density of 1.16 gm/cc in sucrose, K tartrate, or Cs_2SO_4, the nucleocapsid bands at 1.26 gm/cc in these solutes. The nucleocapsid is unstable in high salt concentrations.

The nucleocapsid contains the viral RNA and intact nucleocapsids are sensitive to ribonuclease treatment.

RNA has been extracted from intact virions and characterized by sedimentation in sucrose gradients and electrophoretic migration in polyacrilamide-agarose gels. In addition to the large (10^7 mol. wt.) RNA described by others, RNA with characteristics of cellular ribosomal RNA was also found. Attempts to delineate the cellular site of viral RNA synthesis have not been successful.

Significance to Bio-Medical Research and the Program of the Institute:

A definitive analysis of the structure of the RNA-containing tumor viruses is imperative to the understanding of the chemistry of virus replication.

Proposed Course:

Further characterization of the nucleocapsid, identification of the noted ribosomal-like viral RNA, and continued attempts to determine the site of viral RNA synthesis.

Serial No. NCI-4737
1. Chemistry Branch, OASDC, Etiology
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Analysis of sRNA Species from Beef Brain, Kidney and Liver Tissues

Previous Serial Number: None

Principal Investigator: Dolph Hatfield, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.5
Professional: 0.3
Other: 0.2

Project Description

Objectives:

To compare the patterns and ratios of individual species of sRNA in beef liver, brain and kidney.

Methods Employed:

sRNA is extracted (along with other nucleic acids) from beef liver, brain and kidney with phenol and chloroform. In preliminary experiments, sRNA has been freed from other nucleic acids with DEAE-cellulose. Purified sRNA is charged with one radioactive and 19-cold amino acids and then individual sRNA species are separated by the reverse phase chromatography technique of Kelmers *et al* (*J. Biol. Chem.* *210*:3979 (1965).

Major Findings:

Experiments are still in the preliminary stages.

Significance to Biomedical Research and the Program of the Institute:

Such experiments may elucidate control mechanisms of protein biosynthesis at the level of translation and provide a tool for studying differentiation in different tissues. The phenotypic changes observed during carcinogenesis often involve alterations in the translation of messenger RNA. Understanding the mechanism of translation will lend insight in tumor metabolism.

Proposed Course:

sRNA's of all 20 amino acids from brain, kidney and liver are to be compared. Differences in ratios and patterns of sRNA's in different tissues will be further examined by genetic coding properties and by rates of incorporation into protein.

Serial No. NCI-4701
1. Chemistry Branch, OASDC,
   Etiology
2. Molecular Carcinogenesis
   Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Removal of Messenger-RNA-Like Activity from Rat Liver Microsomes and Effect of Various Carcinogens on this Process.

Previous Serial Number: Same

Principal Investigators: H. V. Gelboin, Ph.D.

Other Investigator: M. E. Weksler, M.D.

Cooperating Units: None

Man Years:
Total: 1.4
Professional: 0.8
Other: 0.6

Project Description

Objective:

To study the effects of various carcinogenic agents on messenger RNA level in cell cytoplasm.

Methods Employed:

Cell-free amino-acid incorporation system using ^{14}C-labelled amino-acids, sucrose gradient centrifugation, spectrophotometry, gel electrophoresis, thin-layer and column chromatography.

Major Findings:

Carbon tetrachloride, in a highly specific manner, removes messenger RNA from rat liver microsomes without significantly affecting the activity of microsomes in protein synthesis, directed by added synthetic messenger RNA, polyuridylic acid.

Significance to Biomedical Research and the Program of the Institute:

The stability of mRNA may be altered during carcinogenesis. Knowledge about the mechanism of mRNA removal during protein synthesis, and the factors controlling mRNA stability are of importance since in most tumors there is an alteration in mRNA stability.

Proposed Course:

To determine the product of mRNA degradation or inactivation during protein synthesis. To study the mechanisms regulating mRNA stability.

Publication

Weksler, M. E. and Gelboin, H. V. Carbon Tetrachloride-Induced Loss of Microsomal Messenger-Ribonucleic Acid Activity. Biochim. et Biophys. Acta, 145: 184-187 (1967).

Serial No. NCI 4703
1. Chemistry Branch, OASDC
Etiology
2. Molecular Carcinogenesis
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Biochemistry of the Initiation Process of Tumorigenesis by 7,12 Dimethylbenzanthracene (DMBA) and Its Inhibition by Actinomycin D.

Previous Serial Number: Same

Principal Investigators: J. S. Wortham and H. V. Gelboin, Ph. D.

Other Investigator: R. Bates

Cooperating Units: Biology Branch, Etiology, NCI

Man Years:
Total: 0.6
Professional: 0.3
Other: 0.3

Project Description

Objectives:

To determine the biochemical mechanism of skin tumor cell initiation and the mechanism by which actinomycin D inhibits the initiation of skin carcinogenesis.

Methods Employed:

Special techniques of chemical isolation and density gradient centrifugation to isolate DNA free of cell contaminants. The characterization of DNA by spectrophotometric and radioactive tracer techniques and its localization *in situ* by radioautography.

Major Findings:

The finding of a parallel between the inhibitory effect of actinomycin D on DNA synthesis and its inhibitory effect on tumor formation suggest that this agent is inhibiting tumor cell initiation by blocking the DNA synthesis and that tumor initiation may require DNA synthesis a cycle of cell division.

Serial No. NCI 4703

Significance to Bio-Medical Research and the Program of the Institute:

The establishment of the requirement of tumor cell initiation for DNA synthesis is important to our understanding of tumorigenesis. A knowledge of the intimate molecular events may allow application of techniques that will prevent or lower the incidence of carcinogenesis.

Proposed Course:

To examine the nature of carcinogen-induced changes in cell culture where the biochemical and molecular events can be more easily examined as compared to *in vivo* systems.

Publications

Bates, R. R., Wortham, J. S., Counts, W. B., Dingman, C. W., and Gelboin, H. V. Inhibition by Actinomycin D of DNA Synthesis and Skin Tumorigenesis Induced by 7,12-Dimethylbenz(a) anthracene. *Cancer Research* *28*, 27-34 (1968).

Serial No. NCI-4717
1. Chemistry Branch, OASDC, Etiology
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Effect of Various Carcinogens, Drugs, and Agents in the Environment on Genetic Transcription.

Previous Serial Number: Same

Principal Investigators: H. V. Gelboin, Ph.D.

Other Investigators: R. G. Wilson, Ph.D. and J. S. Wortham.

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description

Objectives:

To determine the effects of various chemical agents upon the transcription of genetic information into RNA.

Methods Employed:

Sedimentation analyses and separation, isotopic methods, spectrophotometry, electrophoresis, enzymology.

Major Findings:

Previous studies indicated a correlation between the carcinogenicity of various aminoazo dyes in rat liver and the inhibition of RNA synthesis. This was further studied with several carcinogenic nitrosamine derivatives. No correlation was observed between their carcinogenicity and their effect on RNA synthesis. The effect of carcinogenic agents on the pattern of RNA synthesis was studied by separating the RNA species by polyacrylamide gel electrophoresis. The patterns obtained with RNA from carcinogen-treated rats were similar to those of the control rats, indicating that the inhibition of RNA synthesis by the carcinogen is not directed to a single species of RNA but rather that most species of nuclear RNA are inhibited to about the same extent. The enzyme inducer 3-methylcholanthrene stimulates RNA synthesis in isolated nuclei; the pattern of RNA synthesis during MC treatment was examined by polyacrylamide gel electrophoresis. The overall pattern of isotope incorporation was greatest at three hours after the methylcholanthrene was administered. Methylcholanthrene induced unique changes in the pattern of the nuclear 5-10 S RNA.

Significance to Biomedical Research and the Program of the Institute:

The studies suggest that carcinogens may be acting by changing normal genetic transcription and thereby altering cell phenotype. An understanding of the nature of these changes may offer potential for the prevention or the modification of the malignant transformation.

Proposed Course:

To study the mechanism and the nature of carcinogen-induced changes in gene action.

Honors and Awards

Invited to lecture:	Symposium of American Chemical Society, Chicago, Illinois, Sept. 13-18, 1967, "Approaches and Problems in Carcinogenesis."
	Participant - International Symposium on Microsomes and Drug Oxidations, Bethesda, Md., Feb. 16-17, 1968, "Drugs and Microsomal Enzyme Formation In Vivo and in Mammalian Cell Culture."
	22nd Annual Symposium on Fundamental Cancer Research, Houston, Texas, March 6-8, 1968, "Effects of Carcinogens on Gene Action."
	Symposium of Annual Meeting of American Association for Cancer Research, April 11-13, 1968, Atlantic City, N. J., "Effects of Carcinogens on Gene Action."
	George Washington Univ. Medical Center, Dept. of Pharmacology, Washington, D.C., May 9, 1968, "Mechanisms of Enzyme Induction."

Associate Editor, CANCER RESEARCH, 1968-1971.

Committee on Cellular Mechanisms, International Union Against Cancer, 1968-1970.

Reviewer of manuscripts from CANCER RESEARCH, BIOCHEMISTRY, MOLECULAR PHARMACOLOGY, BIOCHEMICAL PHARMACOLOGY, SCIENCE and JOURNAL NATIONAL CANCER INSTITUTE.

Publications

Gelboin, H. V., Wortham, J. S. and Wilson, R. G. 3-Methylcholanthrene and Phenobarbital Stimulation of Rat Liver RNA Polymerase. Nature 214: 281-283 (1967).

Gelboin, H. V. Carcinogens, Enzyme Induction and Gene Action. Chapter in Advances in Cancer Research, 10: 1-81 (1967).

Gelboin, H. V. Carcinogenesis and Gene Action. New Zealand Medical Journal, 67: 111-126 (1968).

Gelboin, H. V. and Conney, A. H. Antagonism and Potentiation of Drug Action. Modern Trends in Toxicology, Butterworth & Co. (Publishers) Ltd., London, England (in press).

Serial No. NCI-4724
1. Chemistry Branch, OASDC, Etiology
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Biochemical Mechanisms Regulating Enzyme Amount in Mammalian Cells

Previous Serial Number: Same

Principal Investigators: D. W. Nebert, M.D., and
H. V. Gelboin, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 2.7
Professional: 1.2
Other: 1.5

Project Description

Objectives:

Various factors, such as age, sex, species, strain, nutrition, hormonal cycle, and exposure to insecticides, drugs or carcinogens, influence the levels of specific enzymes in the intact animal. These factors play a role in determining the susceptibility of an animal to drug and carcinogen action. To study the mechanisms controlling enzyme level. To develop tissue culture techniques in which enzyme induction can be studied within the framework of rigorous biochemical controls. To study the sequence of events whereby specific enzymes are induced, following exposure of cells to the inducer.

Methods Employed:

Cell culture techniques, spectrophotofluorometry, recording spectrophotometry, fluorescent microscopy and radioisotope studies are used in this project.

Major Findings:

Microsomal aryl hydroxylase, an enzyme system involved in drug and carcinogen metabolism, was inducible in hamster embryo cell cultures. The microsomal enzyme has the same characteristics as "mixed-function oxidases" which have been described in mammalian tissues. Six polycyclic hydrocarbons, which include benzo[a]-pyrene, were metabolized by the enzyme. The spectrophotofluorometric determination of hydroxylated benzo[a]pyrene was developed and is sufficiently sensitive to detect 1 μμmole/ml and, hence, has great utility in measuring enzymatic activity of cells grown in culture.

The addition of benz[a]anthracene, or 8 other polycyclic hydrocarbons, to the culture medium produced levels of aryl hydroxylase activity 3- to 40-fold higher than that of control cells. The ability to induce the hydroxylase activity varied in cells of different ages and origins and cells growing at different densities. The mechanism of enzyme induction was studied by measuring the effects of metabolic inhibitors, the concentration of the microsomal cytochrome P-450, and the rates of RNA and protein synthesis. These studies suggest that the induction of microsomal aryl hydroxylase activity involves an activation of specific genes and changes in the translation of messenger RNA.

Significance to Biomedical Research and the Program of the Institute:

Alterations in the control mechanisms of enzyme synthesis and the ability to induce specific enzymes appear to be one of the major abnormalities of cancer cells. An understanding of mechanisms controlling enzyme levels is of importance to our knowledge of the nature of malignant transformation. This enzyme system is involved in carcinogen and drug metabolism as well as the metabolism of a variety of environmental pollutants. Since the enzyme is important because of pharmacological and toxicological considerations, knowledge concerning the mechanisms involved in controlling enzyme level in the organism is relevent to the problems of human drug therapy, toxicology, and response to environmental pollutants.

Proposed Course:

To study the mechanism of enzyme induction and regulation under rigorously controlled environmental conditions allowed by tissue culture methods. To identify those environmental factors controlling enzyme level and their mode of action.

Publications

Alfred, L. J. and Gelboin, H. V. Induction of Benzpyrene Hydroxylase by Polycyclic Hydrocarbon in Hamster Embryo Cells Grown In Vitro. Science, 157, 75-76 (1967).

Nebert, D. W., and Gelboin, H. V. Drugs and Microsomal Enzyme Formation In Vivo and in Mammalian Cell Culture. Proceedings of the International Symposium on Microsomes and Drug Oxidations, Bethesda, Maryland, February 1968 (in press).

Gelboin, H. V. and Nebert, D. W. Effect of carcinogens on Gene Action. Proceedings of 22nd Annual Symposium on Fundamental Cancer Research, Houston, Texas, March 1968 (in press).

Honors and Awards

D. W. Nebert and H. V. Gelboin, participants in International Symposium on Microsomes and Drug Oxidations, National Library of Medicine, Bethesda, Maryland, February 1968.

Serial No. NCI-4733
1. Chemistry Branch, OASDC, Etiology
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Effect of Cigarette Smoking on Aryl Hydroxylase Activity in the Human Placenta

Previous Serial Number: None

Principal Investigators: D. W. Nebert, M.D., and
H. V. Gelboin, Ph.D.

Other Investigators: J. Winker and S. Nyirjesy

Cooperating Units: Department of Obstetrics and Gynecology,
National Naval Medical Center

Man Years:	
Total:	0.3
Professional:	0.1
Other:	0.2

Project Description

Objectives:

To examine the hydrocarbon hydroxylating activity of human placenta from women who smoke cigarettes and from nonsmokers. A positive correlation has been reported (R. M. Welch *et al.*, *Science*, 1968) between the degree of smoking and the presence of hydroxylase activity in the placenta.

Methods Employed:

Spectrophotofluorometry.

Major Findings:

The collection of samples of human placenta and smoking histories are being carried out separately from the aryl hydroxylase assay in a double-blind study. The results are incomplete at the time of this writing.

Significance to Biomedical Research and the Program of the Institute:

The implications of such a study may relate significantly to the higher abortion rate and lower birth weight seen in infants born to smoking mothers. Induction of a carcinogen-metabolizing enzyme in human tissue may be a protective mechanism against environmental carcinogens.

Proposed Course:

To evaluate the report of R. M. Welch *et al.*, and to compare the properties of induced human placenta aryl hydroxylase with that of the induced hydroxylase we have already examined in mammalian embryo cell culture.

Serial No. NCI-4734
1. Chemistry Branch, OASDC, Etiology
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Nuclear RNA Metabolism in the Presence of Actinomycin and Aflatoxin.

Previous Serial Number: None

Principal Investigators: L. R. Younger, M.D. and
H. V. Gelboin, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.2
Professional:	1.2
Other:	0.0

Project Description

Objectives:

To study the changes in nuclear RNA metabolism initiated by the carcinogen aflatoxin and the non-carcinogen actinomycin.

Methods Employed:

Sucrose density isolation of nuclei. Phenol extraction of nuclear RNA. Polyacrylamide gel electrophoresis, spectrophotometry, radioisotopes.

Major Findings:

Both actinomycin and aflatoxin cause a marked inhibition of RNA synthesis with the rapid loss of high molecular weight RNA species from the nucleus. After a high degree of blockage of RNA synthesis with actinomycin the rapidly labeled high molecular weight RNA appears to be converted to lower molecular weight nuclear RNA and cytoplasmic RNA. This conversion is not significantly inhibited by actinomycin.

Significance to Biomedical Research and the Program of the Institute:

Aflatoxin, which is carcinogenic, and actinomycin, which is not, are similar in their acute effects on RNA metabolism; the effects are rapid, and involve an overall inhibition of DNA-dependent RNA synthesis. The high resolution of the newly-developed technique of polyacrylamide gel electrophoresis of RNA permits a detailed examination of the changes in nuclear RNA metabolism following administration of these drugs. Dissimilarities in these changes might be part of the explanation of the carcinogenicity of aflatoxin and thereby provide information relating to the early events of carcinogenesis.

Proposed Course:

To study the effects of carcinogens on nuclear RNA metabolism, degradation and transfer to the cytoplasms.

Serial No. 4730
1. Chemistry Branch, OASDC
Etiology
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Purification and Properties of Nuclear Ribonucleases from Tumor Cells

Previous Serial Number: Same

Principal Investigators: Michael B. Sporn, M.D. and
Harrison M. Lazarus, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 2.6
Professional: 1.6
Other: 1.0

Project Description

Objectives:

To isolate and characterize the enzymes of the tumor cell nucleus which are responsible for the *in situ* destruction of the newly synthesized RNA of the nucleus. To define the functional role of these enzymes in the tumor cell nucleus.

Methods Employed:

Highly purified nuclei have been isolated from a variety of tumors, including Ehrlich ascites, SV-40 virus-induced fibrosarcomas, and mouse mammary tumors. Saline extracts have been made from these nuclei, and the exoribonucleases which degrade RNA have been purified from the extracts. Methods used have been ion-exchange chromatography, gel filtration, and selective heavy metal precipitation. The final enzyme fractions are stable for many months at low temperatures, thus allowing further studies of the mechanism of enzyme action.

Serial No. 4730

Major Findings:

The mechanism of action of the exoribonuclease from Ehrlich ascites tumor cells has been studied in great detail. This enzyme attacks messenger-type RNA from the 3'-OH end to produce 5'-mononucleotides. The enzyme remains complexed with an individual polynucleotide molecule until it has been totally degraded. A new molecular model for this type of continuous degradation has been developed and been tested in the laboratory. It is believed that the enzyme has six principal cationic sites which bind a polynucleotide and keep it bound during the process of degradation. Three new compounds, namely 1) thymidine-3', 5'-di-p-nitrophenylphosphate, 2) thymidine-3',5'-difluorophosphate, and 3) thymidine-3'-p-bromo-acetanildophosphate, have been found to give over 50% irreversible inhibition of the Ehrlich exoribonuclease *in vitro*. These compounds were synthesized for NCI by Ash Stevens, Inc., Detroit, Michigan, under Contract Number PH 43-66-929.

Significance to Bio-Medical Research and the Program of the Institut

The role of the gene action system, both during carcinogenesis and in the ultimate tumor cell itself, is a central theme in a great deal of the research being conducted by the Chemistry Branch, Etiology, NCI. Research on the factors which control gene transcription and translation is among the most basic in all of cancer research. Since messenger RNA is the primary product of gene transcription, it is of utmost importance to know the mechanisms which control both its synthesis and destruction. The present project is yielding data on the enzymes and mechanism of messenger RNA destruction in the tumor cell.

Proposed Course:

To continue studies on the molecular mechanisms whereby messenger RNA is destroyed in the tumor cell and normal cell; to evaluate the role of the enzymes of messenger RNA destruction in controlling the growth and differentiation of both normal and tumor cells.

Serial No. NCI-4738
1. Chemistry Branch, OASDC
Etiology
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Analytical Studies on Nuclear RNA using Polyacrylamide Gel Electrophoresis

Previous Serial Number: Same

Principal Investigators: C. Wesley Dingman, M.D. and Andrew C. Peacock, Ph.D.

Other Investigators: Sylvia L. Bunting

Cooperating Units: Protein Section, Chemistry Branch, OASDC, Etiology

Man Years:	
Total:	0.7
Professional:	0.5
Other:	0.2

Project Description

Objectives:

To study the types of RNA that exist in cell nuclei; to investigate the synthesis and metabolism of these various nuclear RNAs; to determine the intranuclear localization of these RNAs; and to study the extent to which there is species and organ differences in the kinds of nuclear RNA present and in the character of nuclear RNA metabolism.

Methods Employed:

Purified nuclei are isolated by standard techniques. Nuclear RNA is then isolated, purified, and analyzed by electrophoresis in polyacrylamide gels or in mixed polyacrylamide-agarose gels. (See previous Individual Project Report NCI #4731.) Labelled RNA precursors are given *in vivo* when appropriate and the distribution of newly synthesized RNA and stable RNA determined by assaying for radioactivity in gel fractions obtained from the electrophoregrams.

Methods are being developed for obtaining reproducible nuclear subfractions (e.g., nucleoli, chromatin, nuclear ribosomes) so that the RNA content of these fractions may also be studied.

Major Findings:

Many species of nuclear RNA were found which are not detected in the cytoplasm. On the low molecular weight side, the nucleus contained, in addition to a distinct 18S RNA species, several species with mobilities somewhat slower than 4S tRNA and without detectable amino acid acceptor activity. On the high molecular weight side, there were several species of nuclear RNA, with mobilities slower than 30S RNA, which labeled rapidly, unlike cytoplasmic RNA. Newly synthesized 30S RNA and 18S were found to appear in the cytoplasm at the same time, in contrast to results of studies by others. These results also suggested that "35S" and "45S" nuclear RNA's, as defined by sucrose gradient analysis, were not single species, but rather are composed of many components of different molecular weights. This highly complex nature of nuclear RNA makes it difficult to define precursor-product relationships between nuclear and cytoplasmic RNA's.

We have found species but not organ differences in the number and kinds of nuclear RNA's with mobilities between transfer RNA and 18S ribosomal RNA. We have also found species differences in the kinds, and organ differences in the amount, of nuclear RNA's with mobilities slower than 30S ribosomal RNA.

Significance to Bio-Medical Research and the Program of the Institute:

These studies indicate that polyacrylamide gel electrophoresis offers a more discriminating technique for the study of nuclear RNA and its metabolism than has heretofore been available. Furthermore, the technique has allowed comparison at high resolution of the RNA species present in normal versus tumor cells, which, to date, has not revealed significant differences. Finally, several of the Institute's research projects being carried out by other investigators in the Chemistry Branch are utilizing the techniques developed in this project.

Proposed Course:

Nuclear RNA from different tissues of the chick, mouse and rat will be examined. Techniques will be developed and perfected for the isolation of nuclear subfractions so as to enable us to specify which nuclear RNA's are derived from which subfraction. Finally, further studies on the synthesis and metabolism of the various nuclear RNA's are planned.

Publications

Dingman, C. W. and Peacock, A. C., Analytical Studies on Nuclear Ribonucleic Acids Using Polyacrylamide Gel Electrophoresis, Biochemistry, 7, 659-668 (1968).

Serial No. NCI-4739
1. Chemistry Branch, OASDC, Etiology
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Isolation and Analysis of Mitochondrial Nucleic Acids

Previous Serial Number: None

Principal Investigators: Abraham Aronow, M.D. and C. Wesley Dingman, M.D.

Other Investigators: Tsuyoshi Kakefuda, M.D., Ph.D. and Sylvia L. Bunting

Cooperating Units: Protein Section, Chemistry Branch, OASDC, Etiology

Man Years:	
Total:	1.1
Professional:	1.0
Other:	0.1

Project Description

Objectives:

To isolate rat liver mitochondria and purify these organelles to the extent that no detectable nucleic acids from other subcellular fractions contaminate the preparation. To isolate and characterize the RNA and DNA present in the above mitochondria using polyacrylamide gel electrophoresis. To investigate the biogenesis of mitochondrial nucleic acids and demonstrate differences, if any, between the nucleic acids of mitochondria and those found in other parts of the cell. To demonstrate the effect, if any, of hormones and drugs on the metabolism of mitochondrial nucleic acids.

Methods Employed:

Rat liver is homogenized in aqueous buffered sucrose solutions in the absence of magnesium. Mitochondria are isolated by low speed differential centrifugation and multiple washings in magnesium-free medium. Purity of the mitochondrial preparation is assayed by electronmicroscopy. Purified mitochondria are disrupted in detergent and the nucleic acids, having been extracted in phenol, are then fractionated by polyacrylamide gel electrophoresis. When isotopically labeled precursor is used the gel is sliced into 1 mm sections and counted. When DNA is to be isolated in bulk, isopycnic centrifugation in CsCl is used.

Major Findings:

As expected, we found great difficulty in purifying mitochondria; indeed removing microsomal fragments from these preparations is most difficult. Use of non-ionic detergents to differentially dissolve microsomal membrane has not been successful. However, the absence of magnesium in the isolation medium has been found to be a critical determinant in successful purifications. Our preparations are currently approaching the desired level of purity.

The RNA and DNA from the above preparations is generally undergraded and appears to be almost wholly of the 30S and 18S species with little 4S and 5S RNA.

Significance to Bio-Medical Research and the Program of the Institute:

Mitochondria are currently felt to be very primative cytoplasmic structures which appear to husband a cytoplasmic genetic system. These organelles, long known for their metabolic function in the cell have recently been shown to have RNA, DNA and the ability to synthesize proteins *in vitro*.

The RNA of mitochondria may be synthesized on mitochondrial DNA and thereby differ significantly, in nucleotide sequence, from RNA synthesized in the nucleus. Some enzymes which are found in the cytoplasm as well as those in the mitochondria may be of mitochondrial origin. It is important, therefore, to separate and characterize the nucleic acids found in mitochondria. Furthermore, since many important hormones and drugs are known to have a metabolic effect on the cell by their action on the mitochondrion, (*e.g.*, thyroxine), it is important also to establish the effect such drugs might have on mitochondrial nucleic acid metabolism and on mitochondrial enzyme production.

Proposed Course:

We will continue examining various mitochondrial separations until preparations of the requisite purity are obtained. Mitochondrial nucleic acid components will then be isolated and examined using isotopic labeling to characterize their rates of synthesis and subsequent metabolism. We will also compare mitochondrial nucleic acids to those found elsewhere in the cell. Animals treated *in* _vivo_ with hormones and/or drugs as well as animals made hormone deficient will be studied for any apparent effect on mitochondrial nucleic acid metabolism.

Serial No. NCI-4740
1. Chemistry Branch, OASDC
Etiology
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Purification and Characterization of T Antigen from Simian Virus 40-induced Tumors

Previous Serial Number: None

Principal Investigators: Michael B. Sporn, M.D. and
Harrison M. Lazarus, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.4
Professional:	0.4
Other:	1.0

Project Description

Objectives:

To purify the nuclear tumor neoantigen from SV 40-induced fibrosarcomas so that the chemical and functional nature of this cancer-specific protein may be elucidated.

Methods Employed:

Transplantable SV 40 tumors are carried in golden hamsters. Bulk preparations of cell nuclei are made from these tumors, and the T antigen is then purified by saline extraction, ammonium sulfate precipitation, and column chromatography on DEAE cellulose, phosphocellulose, and hydroxylapatite.

Major Findings:

T antigen has thus far been purified several hundred-fold over its concentration in the intact tumor cell. However, the purest material that has been obtained still consists of multiple species of proteins, and it will be necessary to achieve even better purification before many of the desired experiments can be done with the T antigen. Results obtained by immunodiffusion and immunoelectrophoresis suggest that T antigen is a single protein that theoretically could be separated from all other types of cellular proteins.

Significance to Biomedical Research and the Program as a Whole:

The chemical and functional nature of the SV 40 and polyoma tumor T antigens is not presently known, and elucidation of this problem is one of the most fundamental in the entire field of carcinogenesis. The T antigens are apparently cancer-specific proteins, which are not found in non-malignant cells. They are apparently the first new proteins which are synthesized in a cell which is transformed by the oncogenic SV 40 or polyoma viruses, and thus it is of great importance to find out exactly what the role of T antigen is during malignant transformation.

Proposed Course:

To continue studies on further purification of SV 40 T antigen so that a preparation of this material may be obtained which consists of a single species of protein molecule. The chemical and physical properties of this material may then be studied.

Publications

Lazarus, H. M., Sporn, M. B., Smith, J. M., and Henderson, W. R., Purification of T antigen from Nuclei of Simian Virus 40-induced Hamster Tumors. J. Virology, 1, 1093-1095 (1967).

Serial No. NCI-4741
1. Chemistry Branch, OASDC, Etiology
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Effect of Steroid Hormones on Immature Chick Oviduct Nuclear and Cytoplasmic RNA.

Previous Serial Number: None

Principal Investigators: C. Wesley Dingman, M.D.; Bert O'Malley, M.D.; Andrew C. Peacock, Ph.D.; and Abraham Aronow, M.D.

Other Investigators: Sylvia L. Bunting.

Cooperating Units: Endocrinology Branch, General Laboratories and Clinics; Protein Section, Chemistry Branch, OASDC, Etiology.

Man Years:

Total:	1.1
Professional:	0.8
Other:	0.3

Project Description

Objectives:

Previous work by other investigators has indicated that daily administration of diethylstilbestrol (DES) to immature chicks results in a large increase in the size of oviduct and a marked stimulation of protein synthesis resulting in the production of various egg-white proteins other than avidin, while administration of progesterone to these estrogen-stimulated chicks results in the apparent specific induction of avidin synthesis by the oviduct. Therefore, the objectives of this study were to examine, using the high resolving power of acrylamide gel electrophoresis (See previous Individual Project Report NCI-#4731 and current Individual Project Report NCI-#4738), the effect of estrogen and progesterone administration on chick oviduct nuclear and cytoplasmic RNA. It was hoped to learn whether new species of RNA were induced by hormone treatment, or whether there were alterations in the quantity or metabolism of various preexisting RNA's.

Methods Employed:

One group of one-day-old chicks were given daily injections of DES for 18 days; another group got the same treatment but also received an injection of progesterone on the 18th day; while a third group remained untreated. On the 19th day all chicks were killed and their oviducts removed. In some cases these oviducts were incubated *in vitro* with labeled RNA precursors for short periods of time. Nuclear and cytoplasmic fractions were separated and the RNA isolated from each fraction. These RNA samples were then examined by electrophoresis on acrylamide and mixed acrylamide-agarose gels; in some cases the amino acid acceptor activity of the RNA samples was also assayed.

Major Findings:

The major finding has been a large increase in the relative amount of transfer RNA present in the nucleus of oviduct cells from the two groups of chicks receiving estrogen stimulation, and a smaller increase in the relative amount of transfer RNA present in the cytoplasm of these cells. There is also some indication that the newly synthesized nuclear RNA of the unstimulated oviducts is more rapidly degraded and/or converted to lower molecular weight species than is the newly synthesized RNA from the stimulated oviducts.

Significance to Biomedical Research and the Program of the Institute:

This project is aimed at understanding the mechanism by which steroid hormones induce changes in cell function. Steroid hormones have been long known to play an important role in modulating the growth rates and metastatic capabilities of various tumors and for this reason understanding the mechanism of action of steroid hormones is of importance to the program of the institute.

Proposed Course:

The major findings to date will be examined for their reproducibility and experiments on certain control tissues performed to further examine the character of the changes induced in nucleic acid metabolism by DES and progesterone. It is hoped that experiments on the effect of steroid hormones on nucleic acids in other target tissues and in other animals can be performed so as to investigate the possible generality of the findings in chick oviduct.

Serial No. NCI-4742
1. Chemistry Branch, OASDC, Etiology
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
October 10, 1967 through June 30, 1968

Project Title: High Resolution Autoradiographic Study on Mechanism of DNA and RNA Replication in Mammalian Chromosomes

Previous Serial Number: None

Principal Investigator: Tsuyoshi Kakefuda, M.D., Ph.D.

Other Investigators: Emily Anne Olson and Mary Collins

Cooperating Unit: None

Man Years (Computed for the 12 month period):

Total:	1.4
Professional:	0.7
Other:	0.7

Project Description

Objectives:

To investigate the possibility that DNA replication in mammalian cells may be initiated at the membrane (nuclear envelope) similar to certain bacterial systems in which genetic material is apparently linked to the protein of the membrane. In previous experiments it was suggested that early replicating nucleolar DNA triggers RNA synthesis in the nucleolus and appears related to the nuclear membrane. The relation of RNA synthesis to the DNA replication cycle and membrane activity will also be studied. High resolution autoradiography of isolated nuclear macromolecules will be used to provide evidence for any possible multiplicity of DNA replication units present in a single chromosome and to examine the time sequence and direction of polynucleotide chain growth in mammalian cell nuclei.

Methods Employed:

Human amnion cells, HeLa cells and hamster embryo cells are synchronized by blocking DNA replication with excess thymidine

(2.5mM for 24 hrs.). The blockage was released and at selected stages in the cell cycle media containing tritium labeled precursors of nucleic acids are added. The pulse labeled nuclei are then isolated, suspended in a high concentration of salt solution and protein and spread on a surface of water. The nuclear components which were released are then transferred to a grid or glass slide and visualized by shadowing or staining, and sites of incorporation of labeled nucleic acid precursor determined by autoradiography.

Major Findings:

I. ^{3}H-Thymidine Labeled Cells: Electron Micrographs obtained from synchronized cells labeled for 10 minutes after release from blockage of DNA synthesis showed that the majority of the grains (77.2%) were in the outer ring area, particularly close to the nuclear membrane. Ninety percent of the labeled cells showed the ring labeling. The other 10% were homogeneously labeled.

Synchronized cells labeled with ^{3}H-thymidine for 20 minutes showed more grains in the center of the nucleus than those cells labeled for 10 minutes. The outer half of the nucleus of the former cells contained 68% of the grains. The cells labeled for 5 and 10 minutes showed a similar pattern of labeling except the grain density in the former was lower. The striking finding was that the nucleolus associated chromatin was always labeled as rapidly as juxta-membrane chromatin.

Nuclei from unsynchronized cells, labeled under the same conditions, showed even distribution of the grains throughout the nucleus.

II. ^{3}H-Uridine Labeled Cells: Following blockage of DNA synthesis by excess thymidine, ^{3}H-uridine was added and incubation continued for 45 minutes. Grains were almost completely absent from the nucleolus, whereas nucleolar grains from similar pulse labeling in unsynchronized cells were extremely dense. The superficial effect of the excess thymidine seemed similar to that of a low dose of Actinomycin D (but probably occurs by a different mechanism).

When the excess thymidine was replaced by normal media, incorporation of ^{3}H-uridine took place rapidly in the nuclear membrane area and in the nucleolus, i.e., those portions of the nucleus where DNA replication appears to be initiated (see above).

Cytoplasmic labeling, which was also blocked by excess thymidine, resumed in normal media following the recovery of nucleolar RNA synthesis.

III. Structural Organization of the Nucleus: Spread preparations and ultra-thin sections of isolated nuclei showed the chromatin to consist of fine fibrils, uniformly 40Å thick, and arranged in a rosette form which consisted of many loops of a continuous fibril. Frequently grains from ^{3}H-thymidine were found in the middle of the rosettes.

In spread preparations of isolated nucleoli small particles of 150Å to 200Å in diameter were observed. They were either free or associated with the chromatin fibrils. The nature and significance of these particles are not known.

These experiments strongly suggest that DNA replication in mammalian cells is initiated at the nuclear membrane. Both, by model building and by calculation, the expected number of grains per field at the nuclear membrane per ultra-thin section (about 600Å thick) is 2.2 if a single replication site per chromosome is present. High grain count in most of the cells suggested the presence of significantly more than two replication sites in a single chromosome. The fact that blockage of DNA synthesis by excess thymidine surpressed nucleolar RNA synthesis suggests that nucleolar RNA synthesis requires DNA synthesis. The specific template action of nucleolar DNA in terms of nucleolar or ribosomal RNA synthesis has been shown previously by a number of investigators. This might also explain, in part, the transient requirement of DNA synthesis for viral RNA replication in Rous sarcoma and certain murine leukemia systems.

Significance to Bio-Medical Research and the Program of the Institute:

Knowledge of the morphology and biochemistry of DNA replication is important in understanding the control of growth and tumor formation.

Proposed Course of Project:

We plan to extend our study to examine the effect of other experimental environmental conditions including various chemicals, carcinogenic agents, and some viruses on the metabolism of nuclear macromolecules.

The identification of the functional role of the subnuclear and nucleolar components which were observed in spread preparations will be studied further by the use of autoradiography and cytochemical methods.

Serial No. NCI 4745
1. Chemistry Branch, OASDC, Etiology
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Regulation of Specific Function in Cultures of Differentiated Cells.

Previous Serial Number: None

Principal Investigator: Vincenzo Buonassisi, M.D. and
Arthur Friedlander, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	2.0
Other:	0.0

Project Description

Objectives:

To define the molecular requirements for the maintenance of specialized function in culture.

Methods Employed: Clonal line of adrenal tumor responsive to ACTH, classical methods of enzymology, isotope analysis.

Major Findings:

The previous report showed an increase in the amount of synthesis of an RNA fraction sedimenting as 4S in sucrose gradient in adrenal tumor cultures stimulated by ACTH. Previous findings suggest that protein synthesis is an obligatory step in the mechanism of ACTH action and that a DNA mediated step is not required. The assumption has therefore been made that the rate of synthesis of the enzyme(s) stimulated by ACTH is governed by a change in the relative rate of acylation of one of the transfer RNA species present within a set of transfer RNA molecules with the same acceptor activity, but with different anticodons and physical properties. Under certain experimental conditions glutamine is specifically required for a normal response to ACTH stimulation. Transfer RNA from adrenal tumor cultures has been charged with radioactive glutamine *in vitro* and in the intact cell and run on a Kelmers column. Experiments carried out so far have revealed that a new, minor transfer RNA species is charged with glutamine in ACTH stimulated cells; there is also a modification of the chromatographic properties of glutamine synthetase extracted from ACTH stimulated cells.

Significance to Biomedical Research and the Program of the Institute:

The above studies may be important in understanding the mechanism of differentiation and response of a tissue to a specific hormone. Tumor tissue is one in which regulatory mechanisms are abnormal and the regulation of response to specific hormones may lend insight into the abnormal regulation observed in tumor cells.

Proposed Course:

To continue the above studies on the mechanism of ACTH action and differentiation.

Serial No. NCI-4718
1. Chemistry Branch, OASDC, Etiology
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Disc Electrophoresis - A Study of the Fractionation of Protein Mixtures on Cylindrical Columns of Polyacrylamide Gels

Previous Serial Number: SAME

Principal Investigator: Arthur T. Ness, Ph.D.

Other Investigators: Jullia V. Pastewka

Cooperating Units: None

Man Years
Total: 1.5
Professional: 1.5
Other: 0

Project Description

Objectives:

Electrophoresis in gels in general has permitted the resolution or separation of protein mixtures into a larger number of entities than previously possible. Polyacrylamide gels have several advantageous properties for the improved electrophoretic fractionation of protein mixtures. There are two broad objectives: (a) Study of the variables involved in fractionation of protein mixtures; (b) Identification of the fractions and their biochemical significance.

Methods Employed:

1. The disc electrophoresis technique and principles as developed by Ornstein and Davis.

2. Use of other fractionation techniques, such as chromatography, ultracentrifugation, etc., to obtain pure unaltered protein entities to establish identification of the fractions obtained in gel electrophoresis.

Major Findings:

1. A study is in progress on the use of disc electrophoresis to detect and to identify hemoglobin variants in red cell hemolysates. The analysis of these variants is important for diagnosis in certain disease states, and also in

studies of the biochemical genetics of hemoglobin synthesis. The principal known hemoglobins are readily separated. However, the variants C, E, and A_2 have not yet been satisfactorily resolved from one another. The photochemical sensitivity of hemoglobins was found to preclude the photopolymerization of acrylamide gels, and a report is in preparation concerning the effect of fluorescent light on the gel patterns of hemoglobins. The study of the gel electrophoresis of hemoglobins will be continued.

2. A study is in progress on the nature of the unusual reaction between hemoglobin and haptoglobins. The reaction is irreversible and proceeds stepwise through the formation of intermediate complexes to the complete complexes of the haptoglobin-hemoglobin combinations. Several of the multiple bands of the haptoglobins 2-1 and 2-2 types were individually isolated for this study, by the careful excision of the unstained protein bands from the disc electrophoretic gel columns. It is hoped that these studies will also help to elucidate the subunit secondary and tertiary structures of the haptoglobins.

The disc gel electrophoretic technique will be used to study the reversible and irreversible dissociation of haptoglobins into subunits.

3. A study is in progress using disc electrophoresis to determine and evaluate the pattern changes for serum albumins in the presence of urea and other reagents which disrupt the secondary and tertiary structures of proteins.

Significance to Bio-medical Research and the Program of the Institute:

The high resolution and sensitivity of the disc electrophoresis technique permit study of the occurrence of genetic variants of protein entities, and also the analysis of subcellular fractions to determine the site or sites of protein synthesis and a study of biochemical factors involved in protein synthesis.

Proposed Course:

A study of the variables effecting protein fractionations will consist of the following: Concentration of polymer-effect on pore size or sieving properties of the gel; nature of the gel (degree of cross-linking and the chemical nature of the cross-linking copolymer); effect of electrolytes (buffers, pH, and chemical formulation and concentration; neutral salts); effect of non-electrolytes (urea, sucrose, mercapto compounds, etc.); and effect of pretreatment of protein sample (dialysis against H_2O, buffers, salts, etc.); dissociation, subunits, etc.; "denaturation"; chemical modification; and temperature changes.

A future study on identification of bands and their significance will consist of: Use of proteins separated by other techniques (chromatography, gel filtration, etc.); a search for specific markers to locate and identify bands (protein, lipid, carbohydrate, enzyme, metals, etc.); the improvement of generalized protein dye-staining techniques; the study of protein-protein interactions (dilution or concentration of protein mixtures and changing ratios of principa fractions of human serums).

Serial No. NCI-4720
1. Chemistry Branch, OASDC, Etiology
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Haptoglobin Types and Disease

Previous Serial Number: SAME

Principal Investigator: Andrew C. Peacock, Ph.D.

Other Investigators: Sylvia L. Bunting, Ray H. Ragland, and Roswell A. Reed

Cooperating Units: None

Man Years

Total:	1.0
Professional:	1.0
Other:	0

Project Description

Objectives:

The purpose of this study is to extend and amplify findings relating haptoglobin type to differential susceptibility to leukemia and other diseases.

Methods Employed:

Serums are collected from individuals diagnosed either with specific malignant or other diseases or those classified as being normal. Haptoglobin type determinations are made by electrophoretic techniques. Results are tabulated and compared.

Major Findings:

1. Additional Normal Samples. Two hundred thirty-four normal control specimens obtained from the Clinical Center Blood Bank were added to our control series to make a total of 426 normal patients studied. The second set was nearly identical to that observed 3 years previously. The total numbers are as follows: type 1-1, 56; type 2-1, 189; type 2-2, 181. Four hundred five female patients with cancer of the breast were distributed as follows: type 1-1, 57; type 2-1, 196; type 2-2, 142. Detailed statistical analysis indicates that these two populations are statistically different but we are not able to rule out the possibility of some variable other than cancer of the breast.

Haptoglobin subtyping analysis which had been performed on the serums from women with cancer of the breast were compared with similar results obtained from the normal control studies made this past year. Both series show substantially greater frequencies of the "F" gene than we have been led to expect from published studies of others. Because the subtyping technique is relatively new and not commonly employed, we are not certain whether errors in determination of subtype may not be made, and improvements in this technique are being sought.

Physicians in the Clinical Center frequently request serum haptoglobin determination because these values are helpful in some diagnostic problem. We performed 209 such determinations last year.

Significance to Bio-medical Research and the Program of the Institute:

The newer data confirms the tendency observed in earlier reports, namely that a hereditary characteristic conferring susceptibility to various forms of malignancy exists.

Proposed Course:

We plan to study additional patients and other diseases to increase the scope of these findings.

Publications

Peacock, A. C., Gelderman, A. H., Ragland, R. H., and Hoffman, H. A.: Haptoglobin Levels in Serum of Various Strains of Mice. Science 158: 1703-1704, 1967.

Serial No. NCI-4721
1. Chemistry Branch, OASDC, Etiology
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on Electrophoretic Techniques

Previous Serial Number: SAME

Principal Investigator: Andrew C. Peacock, Ph.D.

Other Investigators: Sylvia L. Bunting, Roswell A. Reed, Ray H. Ragland, and A. T. Ness, Ph.D.

Cooperating Units: None

Man Years
Total: .4
Professional: .4
Other: 0

Project Description

Objectives:

1. To study variables in acrylamide technique to improve analysis.

2. To increase understanding of the nature of the separation processes.

3. To extend the range and variety of analytical and preparative uses of this technique.

Methods Employed:

Standard electrophoretic techniques with the use of chromatographic and ultracentrifugal methods to obtain samples suitable for study. Radioisotope counting of ^{14}C and ^{3}H.

Major Findings:

Preparative Electrophoresis. Commercially available units for preparative electrophoresis have been investigated with a view of their applicability in separating proteins and nucleic acids. Numerous defects in the models studied include very low capacity and electroosmotic distortions of the electrophoretic separation.

Applicability of a new stain (1-Ethyl-2-[3-(1-ethylnaphtho[1,2d]thiazolin-2-ylidene)-2-methylpropenyl]naphtho[1,2d]thiazolium Bromide) for the staining of nucleic acids in acrylamide gel. This material, when applied to the gel in a 0.05% concentration in 50% formamide results in the staining of nucleic acids approximately twice as intense as that obtained with our previous stains. The background is rapidly cleared by exposure to light and thus affords a gel mor suitable for scanning purposes than earlier stains.

Findings made during the course of the year on modified types of gels and on relations between mobility and molecular weight are covered in another project report from this Section. Applications to other tissue organelles will be found listed in other Projects.

Significance to Bio-medical Research and the Program of the Institute:

Acrylamide gels offer a promising medium for the resolution analysis of protein nucleic acids, and possibly even cellular organelles. At present, many of the details for optimum utilization remain to be investigated. Further knowledge concerning the fundamental building blocks of the cell is of importance to cancer research. The study of such changes as may occur in these structures during the process of carcinogenesis will be of interest.

Proposed Course:

We propose to investigate the effect of gelation in the presence of urea on the properties of the gel to investigate additional means of providing anti-convective medium for large cellular elements and to conduct experiments on electro-chromatography of substances of biological interest such as proteins and nucleic acids.

Serial No. NCI-4723
1. Chemistry Branch, OASDC, Etiology
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Biochemical Properties of Haptoglobin

Previous Serial Number: SAME

Principal Investigator: Andrew C. Peacock, Ph.D.

Other Investigators: A. T. Ness, Ph.D., Roswell A. Reed, Jullia V. Pastewka, and Sylvia L. Bunting

Cooperating Units: None

Man Years

Total:	1.1
Professional:	1.1
Other:	0

Project Description

Objectives:

To improve reliability of haptoglobin typing and to relate haptoglobin subtyping and structure.

Methods Employed:

Acrylamide gel electrophoresis, chromatography, starch gel electrophoresis in acid urea and mercaptoethanol, and disc electrophoresis.

Major Findings:

Preliminary estimates indicate that the difference in molecular weight between successive members in the polymeric series of the haptoglobins is less than 50,000. These differences were obtained through the use of calibrated Sephadex gel chromatography and by differences in migration rate in polyacrylamide gels of 2 or more concentrations. Several members of the polymeric series of the haptoglobin type 2-1 have been isolated by means of preparative electrophoresis. These preparations will serve for determinations of the molecular weight of the fifth haptoglobin line.

Significance to Bio-medical Research and the Program of the Institute:

Haptoglobin type is believed to be important in the incidence of several forms of cancer and improved methods of determining haptoglobin type are helpful.

Proposed Course:

Studies will be continued to determine whether heterozygous individuals of the type 1F-1S contain dissimilar peptide chains within the same molecule or whether they contain two types of symmetrical molecules. Studies to determine the mechanism of the polymerization will be continued. The chemistry of the unfolding in urea will be studied.

Serial No. NCI-4731
1. Chemistry Branch, OASDC, Etiology
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Electrophoretic Analysis of RNA

Previous Serial Number: SAME

Principal Investigator: Andrew C. Peacock, Ph.D., and C. Wesley Dingman, M.D.

Other Investigators: Sylvia L. Bunting

Cooperating Units: Nucleic Acids Section, Chemistry Branch, OASDC, Etiology

Man Years

Total:	0.7
Professional:	0.7
Other:	0

Project Description

Objectives:

To characterize RNA and DNA by means of their electrophoretic mobility in acrylamide gels and to correlate such observations with other properties of RNA and DNA.

Methods Employed:

Ultracentrifugal sedimentation and density gradients for isolation of RNA; electrophoretic analysis of RNA, assay of radioactivity, photometry, and photography.

Major Findings:

An electrophoretic method has been developed for the analysis of RNAs ranging in size from 10^4 to 10^8 daltons. The method depends on the use of acrylamide gels strengthened with agarose for analysis of the larger RNAs. The resolving power of the method permitted individual characterization of RNAs in mixtures containing multiple species of RNA, without prior purification of each species; RNA molecules which differed in molecular weight by only a few per cent could be clearly distinguished, and the molecular weight of each estimated. This unusual application of electrophoretic methods for the determination of molecular weight is based on the observation that, for RNAs, smaller molecules migrate more rapidly than larger ones. The mobility and the logarithm of the molecular weight are inversely related and this relationship is approximately linear. The molecular weights estimated by this technique, although numerically dependent on values assigned to known RNA standards, are highly reproducible in gels of various composition, and are at present the best means of identification of species resolved by gel electrophoresis. By this means, liver 18S RNA is identified as a doublet of RNAs of 0.66 and 0.62 X 10^6 daltons and the analogous 16S of E. coli as a doublet of 0.58 and 0.54 X 10^6 daltons, while liver 5S RNA has a molecular weight of 38,000 daltons.

The mobility of the several species of RNA is altered depending on the magnesium composition of the buffer. It appears that such changes in behavior may differ characteristically for the several types of RNA. This behavior may provide an additional parameter for the characterization of these RNAs. In particular, it appears that the mobility of 30S RNAs are specifically slowed more than 18S RNA. Further, it appears that fragments derived from these species of RNA may possess this property of the parent molecule.

Significance to Bio-medical Research and the Program of the Institute:

Ability to add new dimensions to the study of RNA under the influence of carcinogenic drugs, viral infection, and "natural" processes may give us new insight into the nature of the malignant transformation.

Proposed Course:

To investigate other parameters that may influence the mobility of RNA and DNA such as changes in the ionic environment with respect to pH, etc. To improve the quantitation of the RNA separated by the electrophoretic techniques.

Publications

Peacock, A.C., and Dingman, C.W.: Resolution of Multiple RNA Species by Acrylamide Gel Electrophoresis. Biochemistry 6: 1818-1827, 1967.

Peacock, A.C., and Dingman, C.W.: Molecular Weight Estimation and Separation of RNA by Electrophoresis in Agarose-Acrylamide Composite Gels. Biochemistry 7: 668-674, 1968.

Serial No. NCI-4732
1. Chemistry Branch, OASDC, Etiology
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Electrophoretic Separation of Proteins of the Endoplasmic Reticulum and Ribosomes

Previous Serial Number: SAME

Principal Investigator: Peter M. Berkman, M.D.

Other Investigators: Andrew C. Peacock, Ph.D., and Jullia V. Pastewka

Cooperating Units: None

Man Years
Total: 1.3
Professional: 1.3
Other: 0

Project Description

Objectives:

To develop adequate methods for the solubilization of proteins of the endoplasmic reticulum and ribosomes, and to separate these proteins by electrophoresis on polyacrylamide gel.

Methods Employed:

Methods included homogenization with motor driven teflon pestle, centrifugation through a discontinuous sucrose gradient to provide a membrane fraction essentially free of ribosomes, solubilization of membrane protein by sonification at neutral or alkaline pH, disc electrophoresis at pH 2.4, 3.9, and 9.4, analysis of tissue components for protein, RNA and phospholipid; Sephadex chromatography on G-100 Sephadex.

Major Findings:

As an extension of the work carried out last year, the electrophoretic separation, carried out at pH 2.4, revealed approximately 15 bands, the electrophoretic pattern was reproducible but some variation; especially with respect to the number of minor bands was usually noted.

When the sonicated material was electrophoresed into gels containing 8M urea in successively decreasing portions of the gel column, the number of bands progressively diminished. In the complete absence of urea, only one major band and two minor bands are visualized.

Gel filtration of the sonicated membrane proteins on Sephadex G-100 at pH 7.6 showed only one peak at the void volume. The addition of 6M urea to both the sample and the Sephadex did not appreciably alter the elution pattern; most of the protein again appeared in the void volume. A similar result was obtained when the sample was eluted with .006M dithiothreitol in addition to 6M urea.

Various fractions from the column eluted with 6M urea were pooled, concentrated and subjected to electrophoresis. The greatest number of bands were to be seen in the fractions comprising the void volume. The composite electrophoretic pattern obtained from the eluant resembled the starting sonicated sample superficially but differed from it with respect to the position of both major and minor components. A rough correspondence between elution volume and mobility was noted.

Significance to Bio-medical Research and the Program of the Institute:

A method of solubilizing the proteins of the subcellular particles and characterizing them by electrophoretic analysis would contribute to an understanding of normal microsomal structure. Since microsomal enzymes are important in the structural alteration of drugs and may contribute to the detoxification of known or unknown carcinogens, such an analysis would facilitate the study of carcinogenic alteration.

Proposed Course:

During the course of this study, it was noticed that increasing concentrations of urea appeared to retard the migration of protein into the gel. Since the use of urea in gels is becoming increasingly prevalent, it would be of interest to pursue this observation further to determine if the presence of urea alters the polymerization of acrylamide so as to produce a tighter, less porous, sieve.

Serial No. NCI-4743
1. Chemistry Branch, OASDC, Etiology
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Electrophoresis of Ribosomes

Previous Serial Number: NONE

Principal Investigator: Albert E. Dahlberg, M. D.

Other Investigators: Andrew C. Peacock, Ph.D., and C. Wesley Dingman, M. D.

Cooperating Units: None

Man Years	
Total:	1.0
Professional:	1.0
Other:	0

Project Description

Objectives:

The purpose of this project is to study the properties of ribosomes and polysomes under various conditions in polyacrylamide gels.

Methods Employed:

Escherichia coli ribosomes and polysomes are electrophoresed in agarose-acrylamide (composite) gels in a magnesium containing buffer. Ribosomal subunits and RNA are electrophoresed in an EDTA containing buffer. Subcellular particles are separated by ultracentrifugation.

Major Findings:

Bacterial polysomes are resolved on composite gels with discrete bands up to octamer size. The bands correspond to polysomes obtained by sucrose gradient fractionation. When polysomes in a gel are electrophoresed a second direction after detergent treatment, 16S and 23S RNA bands are observed for each polysome band.

Ribosomes which migrate in a gel as 70S monosomes in 10^{-2}M Mg, dissociate at lower magnesium concentrations and are resolved into two types of 30S subunits and a 50S subunit in the gel. The two 30S subunits have identical 16S RNA and the 50S subunit has a 23S RNA. The subunits migrate faster than their corresponding RNA's at low magnesium concentrations. In EDTA containing gels the subunits migrate slower than their corresponding RNA's. This suggests that the protein component of the subunit contributes to the conformational arrangement of the RNA in the presence of magnesium.

Significance to Bio-medical Research and the Program of the Institute:

Electrophoretic analysis should provide a new and versatile method of studying the properties of polysomes and the nature of protein synthesis *in vivo* and *in vitro*.

Proposed Course:

The electrophoretic technique will be applied to the investigation of polysomes in normal and neoplastic mammalian cells.

Serial No. NCI-4744
1. Chemistry Branch, OASDC, Etiology
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Role of Ribonucleic Acid Synthesis in the Mechanism of Action of Aldosterone

Previous Serial Number: NONE

Principal Investigator: Peter M. Berkman, M. D.

Other Investigators: Andrew C. Peacock, Ph.D., and Roswell A. Reed

Cooperating Units: None

Man Years
Total: .2
Professional: .2
Other: 0

Project Description

Objectives:

To determine whether the mechanism of action of the hormone, aldosterone, involves RNA synthesis, and to determine whether the pattern of RNA synthesis is altered as a result of the hormone.

Methods Employed:

Homogenization of rat kidney in a Dounce homogenizer, addition of polydextran sulfate to retard the action of ribonuclease, centrifugation of the crude nuclear fraction through 2.1M sucrose, addition of triton to remove remaining cytoplasmic components, extraction of RNA with sodium dodecyl sulfate and phenol, and purification of RNA with ethanol. Similar methods of extraction and precipitation in obtaining cytoplasmic RNA. Determination of pattern of RNA by gel electrophoresis in agarose acrylamide.

Major Findings:

When polydextran sulfate was added to the extraction medium the electrophoretic pattern of rat kidney, nuclear and cytoplasmic RNA revealed RNA species very similar to that seen in other mammalian tissues such as rat liver. The injection of 6 micrograms of aldosterone subcutaneously 2 hours prior to sacrifice yielded an electrophoretic pattern identical with the pattern obtained from the kidney of the control animal. Thus no evidence of new nuclear or cytoplasmic RNA species could be demonstrated at a time when aldosterone is known from previous studies to be active with respect to sodium retention or potassium excretion.

Significance to Bio-medical Research and the Program of the Institute:

The question of the mechanism by which aldosterone exerts its effect is related to the general question of hormonally induced enzyme induction and the factors regulating protein synthesis in the cell.

One of the overall objectives of the Institute is the understanding of the factors regulating protein synthesis and cell growth; an analysis of ribonucleic acid synthesis after the injection of aldosterone would contribute to the general question of hormonal action and the regulation of the synthesis of macromolecules.

Proposed Course:

The pattern of nuclear and cytoplasmic ribonucleic acid will be examined at different times following the injection of aldosterone in addition to the two hour period examined so far.

Isotopic precursors of nucleic acid will be used to determine if aldosterone alters the synthesis of a rapidly labeled component by exposing the rat to brief periods of isotope following the injection of aldosterone. The resulting RNA will then be electrophoresed and the gels cut and examined for radioactivity

If a difference is found between saline-treated controls and aldosterone-treated animals, then a physiologic stimulus such as salt depreciation will be employed in normal animals to try to reproduce the findings observed in the adrenalectomized rats with injected aldosterone.

CONTRACT NARRATIVE
CHEMISTRY BRANCH, ETIOLOGY
Fiscal Year 1968

Office of the Chief

NEW YORK UNIVERSITY (PH 43-66-962)

Title: Studies of Pulmonary Carcinogenesis, Alkylating Agents as Carcinogens and Anticarcinogens in Cancer Induction

Contractor's Project Director: Dr. B. L. Van Duuren

Project Officer(s) (NCI): Dr. Harry V. Gelboin

Objectives: 1) To explore potentially carcinogenic hazards of organic and inorganic chemicals of industrial and environmental origins in animals and to study such relevant parameters of the problem as entry into the respiratory system, removal or retention, and pathologic effects, which may be related primarily or secondarily to the development of lung cancer, and 2) to bioassay for carcinogenicity chlorinated compounds and to study the initiating effects that occur in the presence or absence of promoting agents.

Major Findings: A number of α-Halo ethers were tested for carcinogenicity. The compounds are highly reactive and many are used industrially as polymerization solvents and as intermediates in industrial organic syntheses. One of these compounds, bis-(chloromethyl)ether resulted in squamous carcinomas of the skin in mice and fibrosarcomas in rats. Several monofunctional agents were tested and found to be either weak or inactive as carcinogens while the difunctional alkylating agents were as potent a carcinogen as β-propiolactone. Also, further studies on the inhibition of actinomycin and other compounds on tumor cell initiation have been completed and levels of actinomycin, not exhibiting significant toxicity, were found to inhibit initiation when applied up to 30 days after the carcinogen. Other inhibitors, such as mitomycin, aflatoxin and certain enzyme inducers capable of altering carcinogen metabolism are now being tested. Thus far, acridine orange and d,l diepoxybutane are most effective as inhibitors when applied with DMBA. Also, biological assays of the active tumor-promoting principles of croton oil have been developed.

Significance to Biomedical Research and the Program of the Institute: The materials to be studied include products that are in use industrially and may be found in the environment. The initiation, promotion, and anticarcinogenesis phases will supplement studies of current investigation of tumor-promoting principles of tobacco and tobacco smoke, as well as other studies that are under way to explore the mechanism of initiation of carcinogenesis and the role of multiple factors in the production of cancer of the lung.

Proposed Course: Following the completion of the survey of technological and chemical aspects, a review of the toxicological literature and the study of an industrial plant using reinforced plastic in related materials, the animal exposure studies will be initiated. Additional materials will be added to the study as the work progresses.

Date Contract Initiated: June 29, 1966

Current Contract Level: Sept. 29, 1967 - Sept. 28, 1968: $389,690.

Nucleic Acids Section

ASH STEVENS, INC. (PH 43-66-929)

Title: Synthesis of Purine and Pyrimidine Nucleotides

Contractor's Project Director: Prof. Calvin L. Stevens

Project Officers (NCI): Dr. Michael B. Sporn, Project Officer
Dr. Harrison M. Lazarus, Asst. Project Officer

Objectives: To synthesize nucleotide analogs which will act as irreversible inhibitors of enzymes destroying RNA.

Major Findings: Several new agents have been synthesized by the contractor which irreversibly inhibit the tumor cell exoribonuclease, an enzyme which selectively destroys messenger-type RNA. These compounds include 1) thymidine-3'5'-di-*p*-nitrophenylphosphate, 2) thymidine-3',5'-difluorophosphate, and 3) thymidine-3'-*p*-bromoacetanildophosphate. These agents give approximately 50% inhibition at approximately 10^{-3}M *in vitro*; it is hoped to synthesize significantly more potent agents within the coming year.

Significance to Biomedical Research and the Program of the Institute: This contract is directed at developing a basic pharmacologic agent which can be used to inhibit the destruction of messenger RNA, either during carcinogenesis or in the ultimate tumor cell. Two other agents which interfere with messenger RNA have been of fundamental importance in cancer research, namely actinomycin D (which blocks messenger RNA synthesis) and puromycin (which blocks RNA function). We do not yet have an agent which blocks destruction of messenger RNA. The aim of this contract is to synthesize such an agent, and when a potent one is made, it should find wide application in many laboratories that are studying the etiology of cancer.

Proposed Course: To continue synthetic work, in order to develop more potent agents that will be useful for *in vivo* studies.

Date Contract Initiated: June 21, 1966.

Current Contract Level: June 21, 1967 - June 20, 1968: $76,662.

Summary Report

OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR FOR VIRAL ONCOLOGY

July 1, 1967 - June 30, 1968

Frank J. Rauscher, Jr., Ph.D.

1. INTRODUCTION

The Office of the Associate Scientific Director for Viral Oncology incorporates three Branches which share the responsibility for planning, implementing, and managing all programmed research of the National Cancer Institute on viruses in relation to cancer. They are: (1) the Viral Biology Branch, (2) the Viral Carcinogenesis Branch, and (3) the Viral Leukemia and Lymphoma Branch. Together, scientists within the three Branches cover all of the scientific disciplines necessary for such broad operational management, as well as for studying in depth the specific problems within special disciplines that are essential to the attainment of program objectives. They also participate in or provide guidance for research related to their specialties in many multidisciplinary group activities, such as: (a) comprehensive investigations on specific animal oncogenic viruses and their interactions with hosts and host cells; and (b) the search for viruses in human leukemia and other human cancers which may be etiologically associated with the initiation, and possibly also the continuation of the disease.

The collaborative (contract) portion of the Viral Oncology Program of the Etiology Area, NCI, is composed principally of two research and development program areas -- the Special Virus-Leukemia Program (SVLP) which includes human, and animal studies (SALES) and the Solid Tumor-Virus Program Segment (STVP). The latter program was implemented largely during FY68 and operationally will be implemented within the structure of the SVLP. Two other program areas -- Program Resources and Logistics, and Biohazards Control and Containment -- provide relevant support to the SVLP and STVP.

The following summary is concerned primarily with our principal ongoing program -- the SVLP -- and presents (a) progress highlights from intramural, contract and other program-related laboratories, (b) major scientific and management obstacles to attainment of program goals and objectives, and (c) a discussion of avenues of approach, which in the light of new information, appear to be less fruitful, and which are therefore being curtailed, and a discussion of newer approaches which, based on successful animal system studies, are now being applied to human materials.

Beginning in 1964, the Congress of the United States provided funds to the National Cancer Institute (NCI) for an intensified program in virus-leukemia research. These appropriations have been made to support special efforts and to complement the extensive research funded through the usual mechanism of grants in support of work by other investigators.

Utilizing a new planning approach called the Convergence Technique (L.M. Carrese and C.G. Baker, NCI), an overall program plan aimed at controlling human leukemia was formulated. The funding level for this Program in FY67 has been approximately $16,500,000. The NCI requests for these supplemental funds have been based on the conviction that there exists sufficient knowledge and information and technical capability to plan and implement an intensified and coordinated Program. The main objectives of this Program are: (1) to determine whether viruses comparable to those now known to be associated with avian and murine leukemia are etiological agents of human leukemia, and (2) to develop an effective vaccine or other means for the prevention and/or control of human leukemia and lymphoma when such etiological agents are found. The main assumption or working hypothesis on which the overall Program is based is that at least one virus is an indispensable element for the induction (directly or indirectly) of at least one kind of human leukemia (including lymphoma) and that the virus and/or virus genome persists in the diseased individual.

The program plan was approved by the NCI Scientific Directorate on October 6, 1964, and on October 14, 1964, at a meeting of the National Advisory Cancer Council it was unanimously recommended "that the NACC and the NCI Board of Scientific Counselors go on record as enthusiastically endorsing the scientific plan of attack and the scientific management program as outlined to us, and therefore the making of speedy progress in this problem of the causation of acute leukemia and the means of eradicating acute leukemia."

2. SCIENTIFIC ACTIVITIES

The principal laboratories of the Etiology Area, the personnel of which provide the inhouse intellectual and technical motivation, guidance, and thrust for the SVLP are the Branches of Viral Leukemia and Lymphoma, Viral Carcinogenesis, Viral Biology, Epidemiology, and Biometry. In addition, data and guidance from the laboratories of Drs. Robert Huebner, NIAID, Alan Rabson, LCP, John Fahey, LI, Paul Gerber, DBS, and Seymour Perry, Edward Henderson and Paul Carbone, MB, and others have contributed very significantly to the Program.

(a) Overall Progress Highlights

The following are progress highlights, SVLP, prepared for the Director's (NCI) presentation to the Congressional Budget Hearings, March 1968:

1. DISCOVERY OF NEW ANIMAL CANCER VIRUSES AND DETECTION OF SIMILAR VIRUSES IN HUMAN CANCERS -- During the past year 6 important new animal tumor viruses were discovered. There are now over 60 viruses which are known to cause virtually all kinds of cancer in every major group of animals, including subhuman primates. The direct application of this laboratory know-how to the human disease has revealed that virus-like particles primarily of 2 different types can be detected and in some cases isolated from patients of different countries who are afflicted with different types of cancer. Particles of one kind are identical with

those known to cause leukemia in laboratory animals. Particles of the other type are similar (but not identical) in size, shape and chemical makeup to herpes viruses known to cause fever sores or shingles in man, and cancers in some animals.

This is important because the repeated detection and isolation from patients of viruses similar to those known to cause cancer in animals is in itself indirect suggestive evidence of virus involvement in human cancer and is prerequisite to the growth of larger quantities of virus in the laboratory for crucial characterization studies and for the development of diagnostic antigens and potential test vaccines.

2. RECOVERY OF VIRUS FROM HUMAN LEUKEMIA AND LYMPHOMA TISSUE CULTURES -- We have firmly established the growth of tumor cells in the laboratory from 180 different patients with leukemia, lymphoma and other cancers. Most of these tissue cultures were derived from children and adults of Africa, Australia, England, Japan, New Guinea, Sweden and the United States suffering with leukemia or lymphoma. A herpes-type virus has been detected and isolated from at least 80 of these human cell cultures. We can now grow industrial quantities of these tumor cells and recover large quantities of purified and concentrated virus.

   This is important because we can now grow those quantities of living human cancer cells in the laboratory which are necessary for the isolation and definitive characterization of large quantities of purified and concentrated virus. This virus material is needed to determine its ability to induce cancer in animals and to conduct comparisons of similarities and differences to other human viruses and to animal viruses known to induce cancer in animals.

3. NEW METHODS TO DETECT AND ISOLATE VIRUSES -- Largely during the past year 5 new and highly sensitive methods have been developed for the detection and recovery of tumor viruses from animal cancers. (Helper viruses; co-cultivation of cancer cells with normal cells; DNA - RNA homology; T-antigen and other serological techniques; and centrifugal concentration.)

   This is important because these techniques are directly and immediately applicable to the detection and rescue of viruses in human cancers and, because of their high sensitivities, will dramatically enhance our ability to detect and recover very small amounts of complete or incomplete forms of the virus in human cancer materials.

4. HERPES-TYPE VIRUS "CAUSES" INFECTIOUS MONONUCLEOSIS -- The herpes-type virus which has been isolated from a large number of cancer patients has been reported to cause infectious mononucleosis (a leukemia-like disease) of young adults in this country and presumably throughout the world.

This is important because it shows that a virus repeatedly isolated from human cancer patients is capable of inducing rapid and for a time uncontrolled growth of the same kind of human cells that are involved with leukemia. These findings are also important because they should provide the means to identify people (high school and college age students) who are at high risk to infectious mononucleosis and the development of test vaccines for the prevention of this debilitating disease.

5. HERPES-TYPE VIRUS ENHANCES THE RAPID AND PROLONGED GROWTH OF NORMAL HUMAN CELLS IN THE LABORATORY -- It has been extremely difficult to grow normal human blood cells in the laboratory. However, when the herpes-type virus isolated from human cancer materials is added to human blood cells, such cells begin to grow very rapidly for long periods of time. Some of these cells show the same kind of chromosome damage characteristic of blood cells recovered from cancer patients.

   This is important because it shows that this virus can induce rapid proliferation of human cells in the laboratory, a phenomenon which mimics the rapid and uncontrolled growth of tumor cells in cancer patients.

6. POSSIBLE RELATIONSHIP OF HERPES-TYPE VIRUS TO MALIGNANT LYMPHOMA OF AFRICAN CHILDREN -- The same virus which appears to cause infectious mononucleosis in the United States has been isolated from the tissues of 25 of 26 African children afflicted with a malignant lymphoma. It has also been shown that 100% of African children with this disease have high titers of antibody to this virus, whereas less than 50% of normal children living in the same area have antibodies to this virus.

   This is important because we can now ask very specific questions, the answers to which should be available within this year and which will significantly help to determine whether these herpes-type viruses are causatively related to this lymphoma and perhaps to other human neoplasms as well.

7. IDENTIFICATION OF TARGET CELLS FOR INFECTION WITH HTV -- During this year an improvement in the specificity of a technique for determining the presence or absence of the herpes-type virus associated with Burkitt lymphoma tissue culture isolates has been made. With this improved immunofluorescent technique a consistent correlation has been found between positive staining and the presence of virus particles as determined by electron microscopic examination. The herpes-type virus is usually detected in a small fraction of cultured human white blood cells, and sometimes is not detectable in some permanent cultures. Concentrates of the virus were shown to infect a large proportion of virus-free cells under appropriate conditions. In the initial stage of infection virus was absorbed onto the surface of susceptible cells. Studies showed absorption to some lymphoid cells found in spleen and lymph nodes (but not in the thymus) from humans and rhesus monkeys.

This is important because by a relatively simple test it can be determined which cells are the likely "target cells" of the virus and which are not. It is now possible to conduct immunological studies in a defined system and to further characterize the agent(s). Furthermore, the potential value of the monkey in studies with this agent is indicated.

8. HERPES-TYPE VIRUSES MAY CAUSE CANCER IN LOWER ANIMALS -- Herpes-type viruses appear to produce kidney carcinomas in the frog and leukemia in chickens. These viruses have also been recovered from a large number of chimpanzees and from diseased tissues of 2 out of 2 rhesus monkeys which developed leukemia following irradiation or inoculation with certain chemicals.

   This is important because it indicates that herpes-type viruses may induce cancer in animals including subhuman primates and may for the first time provide controllable laboratory systems, studies of which will provide information directly applicable to man. These findings are also important because they indicate the existence of a subgroup of herpes viruses with a special affinity for lymphoid cells of subhuman primates as well as of man. Subhuman primates thus may constitute a potential reservoir of virus from which transmission by blood-sucking insects may be possible.

9. ANIMAL LEUKEMIA AND SARCOMA VIRUSES PRODUCE "CANCER" IN HUMAN TISSUES GROWN IN THE LABORATORY -- Newly developed methods, described above, have made it possible to show that animal cancer viruses can infect and in some cases transform normal human cells into cancer cells. Such transformed human cells will induce malignant cancers when inoculated into hamsters.

   This is important because it shows very clearly and directly that human cells are susceptible to the cancer producing effects of viruses and suggests that some human cancers may be induced by viruses transmitted to man from his animal pets or from animal products (meat, milk, human vaccines produced in animals, etc.).

10. SOME HUMAN "COMMON COLD" VIRUSES PRODUCE CANCER IN ANIMALS. DO THESE VIRUSES ALSO PRODUCE CANCER IN MAN? -- Approximately 10 different human adenoviruses which commonly infect and produce mild respiratory disease in man have been shown to induce cancers in laboratory animals. These viruses and their specific antigens have already been produced in the large quantities required for definitive study of materials from human cancer patients.

This is important because we can now select human cancer patients with tumors of the type induced in laboratory animals with these viruses and hopefully can then determine whether these viruses were responsible for the induction of these cancers in man.

11. PASTEURIZATION KILLS CANCER VIRUSES -- There is good suggestive evidence that certain cancers of the cow are caused by viruses and that these viruses may be present in milk. Other reports have indicated that people who drink unpasteurized cow's milk may develop a higher than "normal" incidence of leukemia and lymphoma. Recent studies have shown that currently used pasteurization methods kill animal cancer viruses deliberately added to milk for these tests.

    This is important because it shows that human babies and adults appear to be at no risk to infection and disease induction by active cancer viruses which might be present in unpasteurized cow's milk.

12. INSECTS TRANSMIT CANCER VIRUSES OF ANIMALS -- Various species of crawling and flying insects including mosquitoes can transmit animal leukemia and sarcoma viruses from one animal to another with resulting tumor induction.

    These studies are important because they provide (a) clues on how tumor viruses might be transmitted in nature, (b) added credence to the hypothesis that Burkitt lymphoma of African children is insect transmitted, and (c) a possible means for controlling the incidence of some cancers in a manner similar to the control of malaria following eradication of mosquitoes.

13. ESTABLISHMENT OF A NATIONAL REGISTRY FOR CHILDHOOD CANCER DEATHS -- A registry has been established of all children who died of cancer in the United States since 1960 (22,000 through 1964). Analysis of these records has revealed that (1) no cancers occurred in 7,000 Maryland children immunized against measles in 1961 with a vaccine which was later found to be contaminated with a virus capable of inducing cancer in lower animals; (2) when an identical twin develops leukemia under 6 years of age, there is a high probability (1 in 5) that the co-twin will develop the disease soon thereafter; (3) various aspects of the occurrence of brain tumors in children suggest that a virus or other environmental factor may be related to its causation, and (4) certain cancers occur excessively in children with specific congenital defects.

    This registry is important because it provides previously unavailable clues to the origins of cancer; it identifies children at exceptionally high risk of certain cancers, enabling their physicians to keep a close watch for tumors, the early removal of which may be life saving; and it provides a means for follow-up of children exposed directly or through their mothers during pregnancy to agents suspected to be cancer producing.

14. BIOHAZARDS AND CONTAINMENT -- New concepts in containment facilities have been developed. Sophisticated laboratory units and containment equipment which will be used in the study of leukemia have been evaluated using both physical and biological techniques. Environmental factors which influence the laboratory environment are being elucidated. The mechanisms of cross-contamination and the hazard of potential laboratory exposure to man by some candidate tumor agents have been demonstrated. Other laboratories that are participating in the Special Virus-Leukemia Program have continued to receive consultation directed towards improving the control of the laboratory environment.

> This is important because it is essential to minimize potential exposure to man by candidate tumor and leukemic agents and the validity of experimental results is dependent on the maintenance of environmental control of the research laboratory. A candidate tumor agent has been demonstrated to be capable of crossing species barriers. Murine leukemia viruses which have been studied in the airborne state retain their infectivity. These findings attest to the urgency of providing safe and controlled environments for conducting leukemia research.

15. COLLECTION OF CLINICAL SPECIMENS AND EPIDEMIOLOGIC DATA ON BURKITT LYMPHOMA IN AFRICA -- Projects are funded for collection of clinical specimens and epidemiologic information from children suffering from Burkitt's lymphoma in Ghana, Nigeria and Uganda. These children are located and brought to the medical centers for therapy, at which time biopsies and sera are obtained. The patients are followed after treatment and epidemiologic information and sera are obtained from families and neighbors. Seventy new patients have been seen and clinical specimens obtained for immunologic and tissue culture studies. Evidence that Burkitt tumor is related to socio-economic status has been obtained.

> This is important because a herpes virus has been isolated from tissue culture cells of Burkitt lymphoma and this virus has been shown to be related to infectious mononucleosis. Mononucleosis has a similar relation to socio-economic status. The information and specimens obtained in Africa will be used to determine the exact relationship between these diseases.

16. PROGRESS IN PRODUCTION AND DISTRIBUTION OF ONCOGENIC VIRUSES -- The responsibility for virus production and distribution has been centralized in the last year.

> This is important because one office now coordinates all the steps from virus production, monitoring, storage, and issue. With this centralization,

production can be closely geared to the demand. Uniform issue procedures and quality control of product have been established. These steps contributed materially to the efficient provision of resources to research laboratories.

17. SPECIAL PRIMATE PROJECTS -- As part of a study on the response of bushbabies (Galago crassicaudatus) to chemical carcinogens, one animal developed a sarcoma following injection with benzpyrene. Another sarcoma developed spontaneously in a tree shrew (Tupaia glis) which was a member of an experimental breeding colony.

This is important because both of these animals are small in size yet are phylogenetically primative relatives of man. Results of cancer research in these animals should be more applicable to man than results from studies on more distantly related normally used laboratory rodents.

A successful breeding colony of bushbabies has been established.

This is important because it is one of the smaller sized primates and is much more economical to maintain in the laboratory than the now more frequently used rhesus monkey, for which it may be a substitute.

(b) Program Areas

HUMAN LEUKEMIA ETIOLOGY & PREVENTION

Drs. R. A. Manaker, A. J. Dalton, M. A. Fink, & R. A. Miller

and

SPECIAL ANIMAL LEUKEMIA ECOLOGY

Drs. J. B. Moloney & M. A. Chirigos

The Human Leukemia Etiology and Prevention program area is the performance of integrated research and development efforts directed toward the prime objective--prevention of human leukemia by production of an effective vaccine for human leukemia and/or other control methods of virology. An essential target in moving toward this objective is the successful growth of large quantities of human leukemia virus in tissue culture for the immunologic studies requisite to vaccine development. Of equal importance are studies designed to improve the detection of specific biologic activity of human candidate virus preparations for selection of specimens for additional work-up, for monitoring tissue culture virus propagation attempts, for monitoring biohazard work, by enhancing the sensitivity of present laboratory indicator systems and/or the development of new test systems. Provision is being made to increase the capacity for screening large numbers of human leukemics for the selection of the most favorable patients and materials for virus isolation and propagation studies, by the further development and increased production of required reagents, and by increasing the number of trained personnel.

The main research emphasis of the Special Animal Leukemia Ecology Studies (SALES) program area has been inquiry into the nature of animal leukemias and their possible relationship to man. "This group is primarily concerned with the extension of pertinent studies to determine whether viruses are etiologically associated with the induction and continuation of leukemia in animals, with emphasis on those with which man is intimately associated. Specific research projects developed by this group are designed to: (a) detect, isolate, propagate and recover leukemogenic viruses of high purity and potency from cattle, dogs, cats and other species; (b) identify and characterize these viruses biologically, chemically and physically; (c) in particular to determine their host range, and their antigenic and morphologic relationships to other leukemia viruses including candidate viruses of man; (d) develop _in vivo_ and/or _in vitro_ bio-assay and monitoring systems for quantitation of disease induction and viral replication; and (e) in collaboration with the Epidemiology and other program segment working groups to determine what relationship, if any, exists between leukemia in man and, contact with animals and/or consumption of animal products."

The reason and justification for including a SALES effort in a program designed to determine etiology and means of preventing _human_ leukemia and lymphoma, is to ascertain whether known or suspect _animal_ leukemia viruses are also capable of inducing these diseases in man. This consideration is based largely on: (a) the knowledge that many "animal viruses" can replicate and/or induce disease in man; and (b) published reports that certain groups of people, following close and prolonged contact with animals or animal products, apparently develop a higher incidence of malignant disease than people of "normal" populations. Whenever it is established that leukemogenic viruses of man are predominantly from man, and that such human viruses (rather than antigenically related animal viruses) can be used as safe and effective antigens, then most of the SALES effort will be substantially modified or dropped from the Program.

There are now over 60 viruses which are known to cause virtually all kinds of cancer in every major group of animals, including subhuman primates. The direct application of this laboratory know-how to the human disease has revealed that virus-like particles primarily of 2 different types can be detected and in some cases isolated from patients of different countries who are afflicted with different types of cancer. Particles of one kind are identical with those known to cause leukemia in laboratory animals. Particles of the other type are similar (but not identical) in size, shape and chemical makeup to herpes viruses known to cause fever sores or shingles in man, and cancers in some animals. This is important because the repeated detection and isolation from patients of viruses similar to those known to cause cancer in animals is in itself indirect suggestive evidence of virus involvement in human cancer and is prerequisite to the growth of larger quantities of virus in the laboratory for crucial characterization studies and for the development of diagnostic antigens and potential test vaccines.

Since no DNA virus has yet been found to induce leukemia or lymphoma, and since all of the known leukemia and lymphoma viruses are of the same subgroup of RNA agents, it would appear logical, from the animal prototype diseases, to concentrate the search for a counterpart human agent on viruses of the medium-sized, membrane-bound, RNA variety. However, at the present stage of knowledge and experience, it would be unwise to exclude viruses of any type as potential candidates for leukemogenesis simply because no member of the group has thus far been discovered which induces the usual forms of leukemia or lymphoma. The facts that the medium-sized, membrane-bound, RNA viruses of avian leukemia can cause kidney carcinomas as well as leukemia and that fibrosarcomas of nearly identical gross and microscopic morphology can be induced in hamsters with both polyoma (DNA) and Rous sarcoma (RNA) viruses further caution against stereotyping all oncogenic virus-host interactions on the basis of the usually predictable tumor responses of laboratory animals to laboratory strains of tumor viruses.

After successful propagation of a virus in sufficient quantities to support its further investigation, the next step in the search for viruses associated with human leukemia and lymphoma is the demonstration of the capacity to induce neoplasia in some laboratory test-animal or tissue culture system. Testing in animals of the homologous species is impractical. Although the production of neoplasia in other animals would not constitute proof for oncogenic potential in man, it would demonstrate that this basic property is possessed by the virus and would represent important evidence to support the possibility of a similar action in man. In like manner, the induction of *in vitro* cytoneoplasia in human or other cell lines would provide valuable supporting evidence, but would not alone constitute proof for oncogenicity of the agent in man.

The problems of testing for oncogenicity in animal and tissue culture systems have been described by many authors. The induction of leukemia in mice, inoculated when newborn with specimens from human leukemia, has been reported by investigators from 3 different laboratories, but others have failed to confirm this finding. Approximately 700 primates mostly rhesus monkeys, but including animals of 9 species (cynomolgus, African green, baboon, pig-tail macaque, stump-tail macaque, Galago, Bonnet, marmosets and chimpanzees), have been inoculated when newborn with specimens from human leukemia and lymphoma by investigators both at the National Cancer Institute and at other institutions collaborating in the Special Virus-Leukemia Program of the National Cancer Institute. No neoplastic responses have been observed thus far. However, only about 220 of these animals are more than 1 year of age, and the oldest are only about 4 years old. If a leukemogenic agent is responsible for the age-peak which occurs at 3 to 4 years in human childhood leukemia, and if susceptible primates approach the human host in time-to-induction of disease, the primate animals would have to be observed for several additional years before their responses could be interpreted as definitely negative.

The Herpes-Type Virus -- The virus or group of viruses most persistently detected and isolated from human leukemia and lymphoma materials (particularly Burkitt lymphoma) is a Herpes-type virus (HTV). Morphologically, the particle is identical to known members of the Herpes group including Herpes Simplex, herpes Zoster, and cytomegaloviruses. Although much of the current intensive virological studies on Burkitt lymphoma concerns the HTV, it is important to note that Herpes Simplex, vaccinia, Bunyamwera, an unidentified agent and Reo viruses, in addition to HTV, have been detected in materials derived from Burkitt lymphoma patients. It is of interest and possibly significant that only HTV has been detected or isolated from American leukemia patients and from Burkitt lymphoma patients studied in the United States. Of the above agents and in addition to HTV, Reo viruses, particularly type 3, appear to be most uniformly present in Burkitt lymphoma patients. In one study, Reo viruses were isolated from biopsy material from 25 of 90 cases of Burkitt's tumor in Uganda and Tanzania whereas this virus was isolated from only 1 of 21 other patients with non-Burkitt tumors. Neutralizing antibody studies using type 3 Reo virus have shown that there is a "relationship" between this virus and Burkitt's tumor. Antibody was found in 53 of 72 cases of Burkitt's tumor, but in only 12 of 65 controls consisting of a variety of other tumor cases in normal children. Comparable results were obtained using hemmaglutination-inhibition tests on another series of serum specimens. Stanley _et al_. reported the induction of lymphomas in mice following their inoculation with spleen cells from a mouse presenting with chronic lesions induced after neonatal Reo virus 3 infection. The disease appears to be a manifestation of an autoimmune disease, although some features are reported as resembling Burkitt's tumor. These preliminary results and the report by Parker _et al_. on the isolation of Reo 3 virus from mosquitoes, while suggestive of a more than casual relationship with Burkitt's tumor, must obviously await confirmation.

It seems clear from the studies of Kajima and Pollard and Huebner that mice of all strains carry viruses capable of inducing leukemia and lymphoma. Since these leukemia viruses are known to be helpers for at least one of the 2 known murine sarcoma viruses, it would appear unwarranted to ascribe an etiological role to a human isolate of a Reo virus and the induction of a lymphoma in a laboratory mouse.

The most recent finding of apparent high significance to the SVLP was reported in January by Henle _et al_. who presented convincing evidence that at least one of the herpes-type viruses isolated from a Burkitt lymphoma tissue culture is etiologically related to infectious mononucleosis. These data have already been confirmed in total or in part by investigators of 3 other laboratories working within the Special Virus-Leukemia Program. At the very least these data suggest that a DNA herpes-type virus is capable of inducing rapid and, for a time, uncontrolled proliferation of cells of the human hematopoietic system. As with paralytic polio, diagnosed infectious mononucleosis is rare among young adults from lower socio-economic environments. The disease appears to occur primarily in relatively 'well to do' high school and college age students who had not been exposed to the virus as children and who, therefore, prior to disease did not have circulating antibodies to the virus. Of obvious additional interest and importance is the corollary report by Henle that 100% of African children with Burkitt lymphoma have high titers of antibody to this virus, whereas less than 50% of "normal" children living in the Burkitt lymphoma area showed antibodies to this virus. When antibodies were found in the latter group, they were of uniformly low titer. These data now allow us to ask specific questions, the answers to which should significantly help to determine whether these herpes-type viruses are etiologically related to Burkitt lymphoma and other human neoplasms.

Incrimination of HTV seems also strengthened by the apparent relationship of a herpes virus to the induction of renal carcinomas in the frog, and by the recent report of Churchill and Biggs and Burmester that the agent of Marek's disease may be a herpes virus.

The Type "C" Particle -- At the time of launching of the Special Virus-Leukemia Program, which involved the assumption that some human leukemias are caused by viruses, the highest priority was given to the search for human agents of the C-particle type comparable to those associated with avian and murine leukemic diseases. The major factor leading to this decision, as well as to the decision to launch the Special Program, was the finding through electron microscopic studies of virus-like entities resembling C-particles in plasma-pellet fractions from human leukemics and in occasional lymph node biopsies from leukemia and lymphoma patients. Accordingly, the approaches initially introduced in the human studies were based upon the then-existing knowledge concerning oncogenic C-type viruses of animals, and the technological procedures that had been developed for their study.

Experience with animal systems had shown that some specimens from both naturally occurring avian lymphomatosis and "noninfective" tumors induced with low doses of Rous sarcoma virus produced detectable virus after propagation in tissue culture, whereas virus had not been detectable by the most sensitive biological methods in the primary lesions in animals. The principle was similar to that employed for recovering viruses of other types from latent or "silent" infections. But the number of subcultures required was greater, and the time between subcultures was much longer than previously applied to other viruses, the total period involved being measured in many weeks, or months, rather than in days or a few weeks.

Although this approach has yielded a few cases in which C-type particles could be detected, transiently, by EM in human, canine, feline and bovine leukemia or lymphoma cells, success has not yet been achieved in the continued propagation of such entities in tissue culture. Until recently, the experience had been the same for attempts to isolate C-type virus by tissue culture methods from spontaneous leukemias of mice of low-leukemic strains.

Whereas most human studies from this approach have been monitored by electron microscopy, other detection techniques worked out for the animal virus model systems have been, and still are being tried. These include both the reactions of in vitro transformation, and the immunological identification of specific viral antigen. The latter method, of course, is dependent upon the availability of viral antigen for producing specific diagnostic antibody. In the absence of laboratory propagated C-type particles of human origin, current immunological approaches are dependent upon plasma-pellet material containing virus-like C-particles, as identified by electron microscopy.

In vitro transformation has been observed with some leukemic bone marrow specimens, and with specimens from children with mononucleosis, but viral particles have not yet been found in the transformed cultures on EM examination.

The only previously unknown human agent that has been brought to light by these methods, thus far, is the herpes-like virus, or family of viruses, frequently associated with lymphomas, but present also in leukemias and other neoplasias as well as in buffy coat cells of some normal subjects. On the other hand, previously unknown "silent" infections with propagatable C-type viruses resembling the oncogenic agents, but as yet unassociated with any demonstrable biological activity, have been detected by electron microscopy in cell cultures from normal mice of various strains.

In the C-particle animal-virus systems thus far studied, there is a high correlation between the average amount of virus that can be recovered from induced neoplasms (or other specimens of diseased hosts) and the infecting dose of virus. However, there is wide variation about the average, and even at the weakest effective dose levels where little or no virus is recoverable from the tumors appearing in a small fraction of the inoculated animals, an occasional animal is found to show a relatively large amount of recoverable virus in its tumor. Extrapolating the average - and maximal - yield trends to lower dose levels where the frequency of "expected" tumors approaches the natural incidence, it could be "predicted" that an occasional tumor should contain sufficient virus for detection by the methods involved. Assuming that this same relationship would hold, and that naturally occurring neoplasms caused by C-type viruses involve relatively weak doses, it appeared logical to examine large numbers of leukemic cases of human and other animal species in search of the "expected" occasional case (probability unknown, and could be very rare) which might show sufficient virus for detection and further propagation. This approach, which also might be referred to as a needle-in-the-haystack approach, is now being pursued to the extent practicable in studies on leukemic humans, dogs, cats, and bovine animals.

Although a few particles resembling C-type viruses are seen in occasional human cases, as already mentioned, no case has yet been found in which the number of such particles is sufficient to constitute convincing evidence that they actually represent virus. The same is true for the studies on bovine leukemia. On the other hand, two cases of cat leukemia and one of dog leukemia, among numerous cases studied, have been found by separate groups of investigators to show C-particles budding from cell membranes, and present in such numbers as to be considered, unequivocally, as virus. Cell-free extracts of both the cat leukemias induced leukemia when inoculated into newborn kittens, and one of the isolates has been passaged through several additional generations. The limited success represented by these few unusual cases lend encouragement to further efforts along this needle-in-the-haystack approach, at least until more is learned concerning natural and low-dose interactions involving C-type viruses, and more efficient methods can be devised for bringing "hidden" infections to light.

Since the initial launching of the SVLP, a major portion of the effort under this program has been devoted to the study of known C-type oncogenic viruses for developing new knowledge, techniques, and approaches to the human problem. The new approaches now available, owing in large measure to support under this program, are discussed below.

(1) Interactions Between C-Type Viruses -- Within species, leukemogenic viruses can interfere with or enhance responses to sarcoma viruses in both chickens and mice. The former action, long known for avian systems, has served as a basis for indirect *in vitro* detection and bioassays of the noncytopathic, and difficult to work with, avian lymphomatosis viruses. More recently, it has been found that co-infections with leukemia viruses in appropriate concentrations, or superinfection following infection with sarcoma viruses may result in enhancement of oncogenic responses to the latter, rather than interference. Both of these phenomena represent potential highly sensitive criteria for the detection of human leukemogenic viruses, provided that counterpart indicator strains of sarcoma viruses can be found in humans. Program activities in search of such agents are now underway, based upon the additional new knowledge and techniques described in following sections.

(2) Defective Viruses and Helper Virus Activity -- The "defectiveness" of one artificially selected strain of the Rous sarcoma virus has been known for several years, and much new fundamental information on the nature of C-type viruses and their interactions has been derived from studies thereon. More recently a strain of mouse sarcoma virus, the Moloney strain, has been found to be "defective" and also dependent upon co-infection with a leukemia virus for its replication in infectious form. As in the avian system, pseudotypes having different envelope properties corresponding to those of the helper viruses employed can be produced both *in vivo* and *in vitro*, and the defective virus can be used as an indicator agent for the detection of leukemia viruses, through their helper actions.

(3) Persistence of Oncogenic Viral Genomes in Transformed Cells and Rescue Thereof from Noninfective Tumors -- Two recent fundamental discoveries of far reaching significance to the search for tumor viruses are: (a) that the viral genome, or some noninfective form of the virus, persists in cells that have been transformed to malignancy by avian and murine sarcoma viruses; and (b) that the functional genomes, or noninfective forms, can pass between cells in close physical contact, *in vivo* and *in vitro*, in the absence of an infectious process.

Cells of tumors induced in foreign hosts by several of the known strains of avian and murine sarcoma viruses usually do not show replication of the virus in infectious form, probably due to a lack of some enzymatic or other biochemical capability. However, when such foreign, noninfective, cells are transplanted back into natural hosts, or placed in close physical contact with natural host cells in tissue culture, the viral genetic information passes into the natural host cells with the result that the latter biochemically competent cells produce and release infectious virus. If the oncogenic viral genome is of the defective type, the addition of a helper virus is required for the virus production.

Efforts are now underway to apply these new techniques and principles to the search for oncogenic viruses in human leukemias and sarcomas.

(4) Studies on Low-dose Induced Neoplasias as an Approach to the Natural Diseases -- Many of the neoplasms induced by the lowest effective doses of laboratory strains of leukemia and sarcoma viruses are noninfective in the sense that active virus cannot be recovered from them with present approaches and techniques. Such lesions are considered as possible models for the development of methods applicable to naturally occurring lesions from which virus likewise cannot be recovered.

Program activities are now underway for producing such noninfective lesions in "reagent grade" hosts (germfree, and SPF derived therefrom), for studies by various participants in the SVLP in attempts to develop techniques for recovering "hidden" virus from such low-dose interactions. The studies involve not only the new knowledge and technical approaches mentioned above, but also the further investigation of immunological mechanisms in the masking of the "hidden" agents.

SOLID TUMOR-VIRUS

Drs. R. J. Huebner & J. T. Duff

> (ASDVO Note: This section of the report was written by Dr. R. J. Huebner and presents the results of a collaborative effort between his present laboratory in NIAID and investigators (both inhouse and contract) and resources of NCI. In recognition of the extent of his commitment to Viral Oncology and of his imaginative and dynamic leadership, a mutually satisfactory agreement was made between NCI and NIAID to enable Dr. Huebner to lead this program area for NCI during FY68. Dr. Huebner will continue in this capacity in FY69, and in addition, he and some of his NIAID staff will transfer to NCI, at which time he will also become Chief, Viral Carcinogenesis Branch.)

General Comments -- Research approaches to the etiology of human cancer hinge very much on the answer to the following two-part question: What is the fundamental gene alteration in neoplasia (1) repressed or mutant cell genes? This has been the general assumption in the case of most spontaneous cancers and cancers induced by chemicals and irradiation; or (2) the introduction of oncogenic viral genes? Representatives of 4 groups of viruses are established as oncogenic viruses for animals-adenoviruses (human and domestic animal), papovaviruses, poxviruses and B and C type "RNA" myxoviruses.

During the past year basic studies of viral carcinogenesis at the molecular level in experimentally-induced systems have established beyond reasonable doubt that virus inductions of neoplasia, like other effects of viruses on cells are the direct results of highly specific genetic information contained in the DNA or RNA of the specified oncogenic viruses. Thus the etiology of cancers and leukemias clearly caused by viruses is inescapably clear. But what about spontaneous cancers and those induced by chemical or physical agents in experimental systems? This question possibly could be answered within the next year or two in the much studied mouse system since new techniques are now available for detecting covert as well as overt "C" type virus infections of this species.

For six years or more the Laboratory of Viral Diseases, LVD, (formerly the Laboratory of Infectious Diseases) has pursued cancer-virus studies in close collaboration with various areas and segments of the National Cancer Institute. Until FY68, these were chiefly laboratory studies of model systems aimed primarily at the development of in vitro tissue culture techniques for isolating, assaying and characterizing tumor viruses and where this was not possible in virus-free tumors, to develop methods for unmasking and/or otherwise detecting the non-infectious oncogenic viral genomes. By early FY68 these developmental efforts had matured to a point where several techniques were applicable to natural history studies of cancer and leukemia viruses of mice and chickens. Similarly the great promise shown by several newly developed serological, biochemical and tissue culture approaches suggested comprehensive concerted efforts to apply them also to field studies of human cancer.

Consequently, in FY68, the Director of NCI established a Solid Tumor Segment in the Viral Carcinogenesis Branch (VCB) with Dr. Robert J. Huebner, Chief of the Laboratory of LVD, NIAID, as Chairman, and Dr. James T. Duff, Acting Chief of VCB as Vice-Chairman. One of the chief actions of the new Segment's advisory group was to recommend that several newly discovered in vitro diagnostic methods for detecting adenovirus gene expression in adenovirus-induced tumors be utilized as soon as possible to see if similar evidence of adenovirus gene expression could be found in human cancers.

Two highly correlated technical approaches were believed ready for application to the human cancer problem: serological tests for detecting adenovirus specific T or tumor antigens; and biochemical tests for demonstrating adenovirus specific mRNA (see Contract Narrative, St. Louis University, PH43-67-692). Similar procedures were also developed for DNA tumor viruses other than adenoviruses, including SV40 and polyoma viruses.

During the same period the discovery of mouse sarcoma virus by Moloney (NCI) and the development in LVD of other laboratory in vitro tissue culture procedures for isolation and assay of the avian and murine leukemia-sarcoma complex viruses led to collaborative efforts by VCB of the Etiology Area and LVD-NIAID to see if these in vitro techniques were applicable to studies of the etiology of solid tumors as well as of leukemia.

The in vitro techniques included the focus forming (FF) and COFAL tests for avian sarcoma and leukosis viruses, and the FF and COMuL tests for murine sarcoma and leukemia viruses, each of which provided new and simplified test-tube procedures applicable to studies of the role of the "C" type RNA viruses as etiologic agents in naturally occurring cancers and leukemias of chickens and mice. Results of current studies indicate that these viruses represent the most frequent virus infections in these two classes of animals and that they cause solid tumors as well as leukemia and lymphoma. Thus, the new technologies have provided exciting new concepts and new approaches to studies of similar cancers of man and his domestic animals, and plans for applying them to field studies in southern and northern California have already been approved and should be in operation by July of 1968.

DNA TUMOR VIRUS STUDIES

In order to implement the NCI-NIAID collaborative studies of DNA viruses (adenoviruses, SV40, herpes-type), several advisory working groups were established under the auspices of the Solid Tumor-Virus Segment, as follows:

(1) The Adenovirus-Papovavirus Working Group (Dr. R. Huebner, Chairman)
(2) The Herpes Viruses Working Group (Dr. A. Sabin, Chairman)
(3) The Transplantation Antigen Working Group (Dr. K. Habel, Chairman)

The Segment identified the adenovirus T antigen and mRNA test procedures as most immediately applicable to the human cancer problem; resulting recommendations of the Segment led to funding of a number of research contracts with universities and industrial research organizations for carrying out programs designed specifically to implement comprehensive sero-epidemiological and biochemical surveys for evidence of oncogenic adenovirus genome expression in human cancer.

The Serological Study Program -- Two options were available: (1) to test for adenovirus specific T or tumor antigens in human tumor cells by complement fixation and/or fluorescent antibody tests; and (2) to test the sera of cancer patients for specific antibodies to T or tumor antigens representative of the three groups of oncogenic adenoviruses. Since production of purified T and tumor antigen had been achieved in several of the NCI supported research contract laboratories, supervised by VCB and the Solid Tumor-Virus Segment, approach #2 was selected to be carried out in the shortest possible time.

The complement fixing non-virion T and tumor antigens representative of the 12 oncogenic adenoviruses which cause tumors in hamsters (groups A and B), or transform rat cells (group C) were produced in quantity by Drs. Gilden and Kern (Flow Laboratories, Contract PH43-65-634B); by Dr. Klaus Schell (Microbiological Associates, Inc., Contracts PH43-62-200 and PH43-64-941); by Dr. Ariel Hollinshead of George Washington University (Contract PH43-64-515); and by Drs. Herbert Rapp and Berton Zbar of NCI.

Purified adenovirus virion antigens were furnished by Dr. Maurice Green (St. Louis University, Contract PH43-67-692).

Twenty or more human sera representative of each of the major types of cancer, and comparable control sera were collected under Dr. Robert Depue's (NCI) supervision at the M.D. Anderson Hospital on Contract PH43-66-937; thus 390 sera were finally made available for complement fixation (CF) and fluorescent antibody (FA) tests by several collaborating groups.

Dr. Huebner's group in NIAID tested the sera by complement fixation versus T, tumor and purified viral antigens; Drs. Zbar and Herbert Rapp of NCI tested them with the adenovirus type 7 T antigen. The results of the CF tests are now being rechecked by Drs. R. Gilden and J. Kern (Flow Laboratories, Contract PH43-65-634B) who will compile a detailed report on these results. Using the FA test, Drs. Fred Rapp and J. Melnick tested the 390 sera versus T antigens produced under the cover of cytosine arabinoside in monkey kidney cells, and Dr. John Riggs and Dr. Edwin Lennette tested them versus tumor antigens present in hamster and rat tumors induced by oncogenic adenoviruses representative of the three oncogenic groups of human adenoviruses.

The results on the 390 sera collected were in agreement and unequivocal. Less than 4% of all the sera showed reactions to the various representative antigens in CF, and were fewer in the FA tests. Thus there was complete agreement that 96% of the cancer patients and controls contained no antibodies to the virus specific T, tumor and virion antigens of the oncogenic adenoviruses. The few positives in the cancer group were matched by equal numbers in the control group. All the evidence indicated that these reactions were randomly distributed, nonspecific cellular isoantigens, a conclusion supported by the almost complete lack of agreement among the test groups concerning the "positive" specimens.

This study is important because a well controlled large-scale serological-epidemiological study has finally shown that human cancer patients do not show significant antibody responses to the T or tumor antigens representative of the known oncogenic human adenoviruses. Although this result is conclusive with respect to this important and specific question, other more sensitive tests must still be utilized in similar well controlled studies in order to rule adenoviruses out of consideration as causes of human cancer, as follows:

(1) Biochemical Tests for Adenovirus Specific mRNA in Human Tumors -- During 1967 Green and his associates at St. Louis University Institute of Molecular Biology (supported in part on NCI and NIAID contracts) described a new and exciting biochemical-molecular approach based on specific homology and hybridization between purified adenovirus DNA and isotope tagged mRNA of virus-induced tumor cells. This approach appears to have wider application for the detection of adenovirus genes than the serological method described above. In blind collaborative studies with LVD it proved to be 100% accurate when used on T-positive and negative adenovirus induced tumors, distinguishing in every case tumors induced by groups A, B or C of oncogenic adenoviruses, and between adenovirus tumors and tumors induced by other DNA viruses and those produced by RNA viruses. The test appeared to be more "sensitive" than the serological approach.

Dr. Green has also shown that the mRNA-DNA hybridization test is ready to be applied to human tumor material. He has prepared and is preparing on contract large amounts of purified DNA for all of the oncogenic and non-oncogenic adenoviruses. However, in order to complete the studies now planned, Dr. Green's facility at the St. Louis University Institute of Molecular Biology requires additional and more suitable laboratories and personnel; large numbers of specimens representative of all types of human cancer must also be acquired. A contract proposal and plans for obtaining the needed facilities have been approved by the Segment and are being processed in NCI. Efforts are now underway in St. Louis and Los Angeles to obtain the needed human tumor specimens within the next year.

This study program is important because it represents a highly specific method for recognizing covert viral genes in virus-free tumor cells which may prove more sensitive and have wider application than the serological approaches.

(2) Serological Tests for Virus Specific Antigens in Human Tumors -- Two laboratories supported by the VCB and the Solid Tumor-Virus Segment plan to use the FA test to study human cancer cells for evidence of adenovirus T or tumor antigens.

This approach is important because FA studies of experimental tumors suggest that as few as 2 to 10% of the tumor cells may contain T antigen. Such a low degree of involvement would not produce positive results in the CF test, thus suggesting that the FA test will prove to be a more sensitive one for antigen detection.

(3) CF and FA Studies of Human Tumors Must Also be Done for the Presence of T Antigens and Antibodies to Representative Adenoviruses of Domestic Animals (monkeys, cows, pigs, dogs and chickens) which have also been shown to be oncogenic when injected into hamsters or tested for transforming ability in tissue cultures.

This is important because certain adenoviruses and papovaviruses of animals seldom if ever produce tumors in their natural host but do so under experimental conditions in heterologous hosts. Since these viruses have many opportunities to infect any possible oncogenic effects they may have must be studied.

(4) In vitro methods for identifying and assaying for virus specific transplantation or surface membrane antigens in virus-induced and other tumor cells are important because in some clones of tumor cells other evidence of virus specific activity cannot be demonstrated.

RNA TUMOR VIRUSES AS ETIOLOGIC AGENTS OF SOLID TUMORS AS WELL AS LEUKEMIA

The ultimate objective of the VCB-LVD studies of the "C" type RNA viruses, which are natural causes of "solid tumors" as well as of leukemias in mice and in chickens, is to find out if similar neoplasms of man are also caused by such viruses. Since "C" type viruses are rarely, if ever, seen by electron microscope in leukemic or other human cancer tissue, and none have yet been isolated in vitro despite extensive efforts, one must conclude that such viruses either are not involved in the etiology of human cancer or, if they are, the "C" type viral gene in man is seldom if ever expressed to the extent that the complete virus is easily observed or isolated.

It is important to note that this does not mean that such viral genes are absent from human tumors since newly developed in vitro diagnostic methods show that "C" type particles and infectious virus may frequently appear to be absent in avian or murine tumors when in fact the genomes of these viruses are present; the latter are simply not expressed as complete or infectious virus particles. Therefore, while many leukemias, lymphomas and sarcomas of mice and chickens readily reveal "C" type infectious particles (mainly when they occur in vertically infected highly inbred strains of animals), many others do not. Fortunately we developed in FY67 for "C" viruses of both classes of animals highly sensitive tissue culture techniques for demonstrating covert and/or incompetent particles, and even for unmasking totally unexpressed viral genomes. During FY68 these techniques reached a degree of sophistication which permitted extensive natural history studies of numerous large populations of mice at the NIH and the Jackson Memorial Laboratory. We found that several inbred mouse strains such as the BALB/c, C57Bl and others, thought previously to be free of oncogenic viruses because of the virtual absence of spontaneous cancers before 2 years of age actually experienced widespread covert or "masked" infection with the "C" type viruses (described below).

Similarly, non-inbred general purpose Swiss mice (NIH-GP, JAX-SWR) in certain colonies appeared to be virtually free of such viruses until they developed lymphoma or solid tumors very late in life, at which time previously undetectable leukemia-sarcoma viral antigen was suddenly unmasked. On the other hand, non-inbred commercial mice (such as CFl and CFW) were shown to be heavily infected with covert "C" type viruses of the leukemia-sarcoma complex. These paradoxical situations in different mouse colonies apparently reflect selective pressures of breeding practices which have led to segregations of different degrees of genetic susceptibility to "expressed" infection with leukemia-sarcoma viruses.

These observations and the "switch off-switch on" leukemia virus genome behavior (to be described below) and the frequent silent presence of sarcoma viruses RSV(O) and MSV(O) in nonproducer tumors cells are important because they suggest that the apparent "absence" of overt "C" type RNA virus in the leukemias and sarcomas of man may be more apparent than real.

Since efforts to isolate a focus-forming competent sarcoma virus from human sarcomas has not been carried out systematically in new tissue culture systems, and since leukemia virus isolation procedures similar to the COFAL, COMuL and focus-forming tests have not yet been developed for detecting postulated human leukemia viruses, both the judge and the jury are still out concerning the very important question above; namely, are human cancers and leukemias caused by "C" type RNA viruses?

Comprehensive Studies of the Properties and Natural Behavior of Murine Tumor Viruses -- Unlike most of the oncogenic DNA viruses, the "C" type leukemia-sarcoma viruses have long been known to commonly cause neoplasms in their natural hosts under natural conditions; they were known also to be transmitted vertically through the mother and horizontal spread had been demonstrated in chicken populations wherein lymphoid leukosis has often been epidemic.

Although naturally occurring horizontal spread had not been demonstrated in mice, it was highly suspect. Therefore, various modifications of the focus forming (FF) test, the complement fixation test for murine leukemia (COMuL), and the genome rescue test developed during 1966 and 1967 in the Laboratory of Viral Diseases were utilized in efforts to determine the properties and natural history of murine leukemia and sarcoma viruses; much of the work, of course, was based on and patterned after the earlier studies of the similar avian leukosis-sarcoma complex viruses.

The Properties of the "C" Type Murine Viruses -- The myxovirus-like properties of the "C" murine leukemia-sarcoma viruses (MuLV and MSV) were confirmed. Like their avian counterparts, these viruses were shown to have an internal soluble protein coat best characterized as the group specific (gs) complement fixing antigen common to all of the known "C" type RNA viruses of the mouse, and an outer lipoprotein envelope best characterized as the type specific envelope antigen, which is distinct for the different serotypes.

The gs antigen demonstrated by Geering et al. to be released from the virus particle by ether treatment was shown to be like the avian gs antigen in its sedimentation properties; i.e. much lighter than the virus particle. Since it is a common antigen in all naturally occurring leukemia and sarcoma viruses, it provided a simple antigenic basis for the wide usefulness of the COMuL test.

Prevalence of Murine Leukemia-Sarcoma Viruses -- Working in collaboration with many scientists in the National Cancer Institute and at the Jackson Memorial Laboratory, Microbiological Associates, Oak Ridge, Baltimore Biological Laboratories and Flow Laboratories, NIH scientists Hartley, Rowe, Igel, Sarma, Rhim and Huebner in FY68 isolated several hundred "C" type COMuL positive viruses from at least 25 different inbred and general purpose strains of mice. These viruses all fall into at least 5 different serotypes corresponding to the standard strains represented by the Gross (or AKR), Friend, Moloney, Rauscher and Kirsten strains, the specificity of each being determined by their outer "envelope" antigens. However, all isolations from naturally infected mice not exposed to animals experimentally infected with Friend, Moloney and Rauscher viruses proved to be of the AKR or G+ type.

The isolates also all contained the common gs internal antigen and by all physical-chemical measurements appeared to belong to the same species of virus as the standard murine leukemias. Those (40 or more) that have been tested, like the standard strains, serve as helpers furnishing envelopes to the defective MSV genome and, when grown out to high titer, generally cause leukemia when injected into susceptible mice.

Many of the "wild" isolates were from leukemic mice and mice carrying solid tumors but a large proportion were from normal tissues of normal mice, chiefly from spleen and thymus. Virus was regularly isolated from normal embryos and newborns as well as from tissues of adults of the so-called high incidence leukemia strains (AKR, C58, C3HFg). However, similar G+ viruses were also isolated from "low incidence" strains, BALB/c, C3H/HEN, DBA, CF", CF1 and others; the frequency of isolations increased with increasing age. Certain strains of mice such as C57Bl, NIH, SWR(Jax), Alb/2, Swiss and NZB generally failed to yield virus in the COMuL test even when leukemic despite the fact that lymphomas and leukemic spleens contain the virus specific gs antigen.

Horizontal Spread of Leukemia Virus (Airborne?) -- At the Jackson Memorial Laboratory, 100 leukemia free but leukemia susceptible SWR strain mice were exposed to possible leukemia virus infection at 6 months of age by placing the SWR mice in open cages adjacent to cages containing naturally infected AKR and C58 mice of the same age. Between 40 to 100 days later, 4 of the SWR mice developed leukemia and their spleens and lymph nodes were shown to contain the virus specific gs antigen. Leukemia had not previously been found at Jackson Laboratories in 1500 SWR mice observed intensively until death. Similarly, a number of C57Bl six-month old mice following the same exposure developed CF antigen in their spleens, an observation not made previously in the normal mice of this strain. Since none of the mice were able to make intimate physical contact, the apparent spread of leukemia is presumed to have been airborne. Since Dr. Hellman and his collaborators at Ohio State have demonstrated airborne infection of mice by aerosolized Rauscher virus, and we (Annual Report FY67) demonstrated MSV and MuLV in urine, saliva and feces of infected mice, horizontal airborne spread would not seem unlikely.

Naturally Occurring MSV Virus - Nondefective (Competent) MSV -- The original Moloney and Harvey sarcoma viruses (M-MSV and HMSV) were shown by Hartley, Rowe, and Huebner to also contain excess amounts of nonsarcomatous leukemia virus. The M-MSV often required a 2 hit mechanism for replication. Recently we studied the FBJ "virus"--an osteosarcoma inducing virus--isolated by Miriam Finkel of the Argonne Laboratory in 1965 from a bone tumor which occurred spontaneously in a CFl mouse. We have confirmed Finkel's observations and extended them by concentrating and purifying virus so that it now produces bone sarcomas locally within a few days after intramuscular injection of newborn CFl and NIH Swiss mice.

We have shown that the FBJ sarcoma virus is serologically identical and in other ways similar to "C" type mouse leukemia virus which we have found to be highly prevalent in normal CFl mice. The naturally occurring FBJ osteosarcoma virus, like the avian osteosarcoma and fibrosarcoma virus described by Rous (1911-1914), suggests that osteosarcoma and related muscle and fibrous tissue sarcomas can occur independently as competent viruses and is important because it suggests that direct attempts to isolate such viruses in the newly developed in vitro tissue culture systems should be tried.

Studies of "hidden" RNA Virus Genomes in Spontaneous and Chemically Induced Murine Cancer -- Much of the scepticism with which the virus theory of cancer was regarded in the past was due to the fact that many spontaneous sarcomas of chickens, and leukemias and sarcomas of chickens and mice, failed to furnish evidence of infectious virus or of virus particles visible in the electron microscope. More importantly, most lymphomas and sarcomas induced in mice by oncogenic chemicals were also found to be "free" of virus. This was particularly true when mouse strains known to be relatively free of spontaneous lymphomas and cancer were used.

The discovery that avian sarcoma viruses were frequently defective, being incapable of replicating in the sarcoma cell and requiring a competent leukemia virus helper for infectiousness, provided a wholly new and unique view of the avian tumor viruses. When the Moloney sarcoma virus was also shown to be partially defective in induced sarcomas of the mouse and the rat, and completely defective in sarcomas induced in hamsters, and that this lost infectiousness could be restored by a competent mouse leukemia virus, it became obvious that the Moloney sarcoma viruses were pathogenic mutants of leukemia viruses which were able to cause tumors in the absence of domonstrable replication of infectious virus.

These observations offered one possible explanation for the absence of demonstrable virus in many spontaneous and chemically induced sarcomas of mice, which resembled in every respect the sarcomas induced by the sarcoma viruses. However, this did not provide a satisfactory answer for the absence of virus in the spontaneous and chemically induced leukemias and lymphomas in the C57Bl strain, which was most often used. Recently, however, Drs. Hartley, Igel and Huebner, using new techniques, demonstrated that most of such lymphomas have a "C" type virus containing the gs antigen of the murine leukemias.

In a long term collaborative study, using the COMuL test, it was found that leukemia virus could be isolated in NIH Swiss cells, but not in BALB/c cells, from spleens of normal BALB/c mice. Virus was isolated with increasing frequency with age, approaching 80% at 20 months. However, when the BALB/c's developed leukemias and tumors late in life, viruses could no longer be isolated in NIH cells, but were readily isolated in BALB/c cells. Large numbers of isolation attempts have followed this paradoxical pattern, suggesting that while virus is frequently present, it is "switched off" with respect to the host (BALB/c) cells; when viewed in perspective perhaps a quite logical biological arrangement for a parasite to have with its host. Thus in the BALB/c cell the virus is switched off "MuLV(0)" corresponding perhaps to the MSV(0) described by Ting; however for other mouse cells (NIH Swiss) it is "switched on" MuLV(+). However, when leukemia or solid tumors are present in the BALB/c the viruses are now MuLV(+) for BALB/c cells and MuLV(0) for NIH - thus indicating a remarkable change in the indigenous BALB/c virus which takes place rather suddenly after oncogenic transformation occurs. This arrangement may be critical with respect to survival of the BALB/c strain and enormously interesting from a molecular biology point of view.

Another example of the switched off - switched on pattern is seen in the C57Bl mice, which yield no evidence of infectious virus or of gs antigen in their tissues as long as they remain normal; however after spontaneous tumors occur or tumors are induced by irradiation of chemicals, infectious virus can frequently be demonstrated *in vivo*, as reported by Kaplan, and in the COMuL test using a continuous line of C57Bl embryo cells as demonstrated recently by Igel and Huebner (in preparation). Although the amount of infectious virus demonstrated is very low in quantity, the tumor and splenic tissues often contain large amounts of gs antigen. The sudden "switching on" of demonstrable infectious virus at the time of spontaneous or chemically induced tumors suggests that the tumored mice contain a "switched off" MuLV(0) prior to tumorigenesis.

What is the Significance and Importance of These Recent Observations? -- They cannot help but produce radical changes in previous concepts concerning the mechanisms by which chemical carcinogenesis is induced in laboratory mice. Instead of the chemical inducing a repression or somatic mutation of a cell gene it now seems likely that it is engaged in converting a silent leukemia virus gene, MuLV(0), into an active one, MuLV(+). This new hypothesis of chemical carcinogenesis in the mouse system much used for such studies is subject to experimental testing, hence studies of the role of indigenous leukemia-sarcoma viruses both infectious and silent as a factor in chemical carcinogenesis will be intensively explored in FY69.

Studies of Avian and Murine Sarcoma Viruses and Their Implications for Similar Studies of Human Sarcomas -- Pseudotype MSV's -- Approximately 30 pseudotype mouse sarcoma viruses (MSV) have been produced by using newly isolated wild strains of mouse leukemia viruses as helpers for the defective MSV hamster genome (Huebner in 1966). These viruses all induce almost identical fibrosarcomas in mice, showing that their pathogenic behavior is sarcoma genome dependent. However, their serological specificity and host-cell range in vivo and in vitro are determined by the leukemia virus envelope. Thus MSV's have been produced which have broad and narrow host ranges; some go only in one strain of mouse, others in many strains. These observations on mouse sarcoma which follow the same patterns observed for avian sarcoma are important because they increase significantly the possible pathogenic options available to the "C" type leukemia-sarcoma particles.

Demonstration of a "C" Type Virus in a Rat Tumor (Novikoff Ascites) -- Laboratory rats are also much used for chemical and nutritional carcinogenesis studies partly because they are nearly as responsive as mice but also because they are generally believed to be free of demonstrable indigenous cancer viruses. However this may not be the case. Several years ago Rowe, Hartley, and Huebner reported complement fixing test evidence of antibodies to murine leukemia antigens in sera of several strains of commonly used laboratory rats. In 1967, in collaboration with Dr. Sarma (MAI, NCI Contract 941), Drs. Huebner and Hartley initiated a systematic study of rats in an effort to detect either fully infectious MuLV(+) or, as seemed more likely, a silent MuLV(0) or MSV(0) viral genome.

Recently it was noted in Dr. Robert McAllister's group (Childrens Hospital of Los Angeles) that the Novikoff ascites rat tumor contained numberous "C" type particles. This tumor we found contained a CF antigen which reacted with a rat antisera having antibody to the Rauscher leukemia virus. However, there was no reaction with antisera prepared against the gs mouse antigen found in the Rauscher and all other murine leukemia and sarcoma viruses. Subsequent studies show that rats carrying the Novikoff tumor develop antibodies to Novikoff rat cell preparations but do not react with the gs antigen of murine leukemia viruses. This suggests that the "C" type particle in the Novikoff rat tumor is a murine "C" type virus distinct from the mouse viruses.

Also of importance are observations by Drs. Klement and Hartley who have shown that Dr. Kirsten's (University of Chicago) murine-erythroblastosis virus after passage in the rat produces focus formation in rat and mouse cells and various sarcomas including bone lesions in the rat. This virus apparently has an envelope that is serologically different from all the mouse viruses, yet the gs antigen is the same as that observed in the mouse leukemias. The virus is unusual also since it is not defective in the sense that it has a one-hit curve infectivity in tissue cultures and does not appear to contain a COMuL positive leukemia virus in excess of its focus producing activity.

These studies of the rat are important because they indicate that rats may indeed have an indigenous leukemia-sarcoma virus of their own; however, this agent, like that of the C57Bl mouse, may be generally silent or covert, requiring activation by some extraneous carcinogenic factor before infectious virus can be demonstrated. On the other hand, the discovery of an antisera with specific antibodies to a rat leukemia-sarcoma virus may serve the same purpose as the COMuL test, making it possible to demonstrate group specific antigen of a rat leukemia-sarcoma virus.

Search for "C" Type Viruses in Solid Tumors and Lymphomas of the Cat, Dog and Other Domesticated Animals -- The purpose of these studies in VCB is to attempt to adapt the new *in vitro* techniques for avian and murine "C" type leukemia and sarcoma viruses to studies of cancers of larger animals.

Dr. R. Gilden and Dr. J. Kern (Flow Laboratories, NCI Contract 634) are collaborating with Drs. Huebner and Hatanaka and with Dr. Rickard of Cornell University, Dr. Brodey of the University of Pennsylvania and Dr. Chapman, University of Kansas, in studies of canine lymphoma. Although several attempts to confirm Rickard's cell free transmission of a mastocytoma virus in the beagle have not been confirmed, viral particle "bands" have been prepared for more critical studies.

Chapman's canine lymphoma cell cultures were shown to have "C" type particles and interestingly enough such preparations contained antigens identical with the gs antigen of murine leukemia. These possibly exciting observations, however, must be questioned until it is shown that the canine cultures are not contaminated with mouse cells and mouse virus.

Dr. Sarma (MAI, NCI Contract 941) is cooperating with Dr. Rickard, Dr. Theilen (University of California) and others in attempts to develop a COFAL-COMuL like diagnostic test for studies of solid tumors and lymphomas in cats. This approach seems particularly pertinent since Rickard and Theilen have reported cell free transmissions of feline leukemias. They have had subsequent difficulties in establishing serial passages of the virus in free living cats because of intermittent microbial infections. An *in vitro* tissue culture system for growth and assay of the feline leukemia virus would represent a tremendous step towards making field studies possible.

Drs. Sarma and Huebner are conducting similar studies of avian household pets such as the parakeet (budgerigar) which is reported to frequently die (in old age) of a variety of cancers including lymphomas and sarcomas. Since young humans (particularly in recent years) are perhaps more frequently and intensively exposed to parakeets than to any other pets, the postulated leukemia and sarcoma viruses of these animals also require study.

These studies are important because they add another dimension as well as new in vitro techniques to the NCI's SVLP efforts to determine if household pets, all of them subject to considerable cancer and leukemia, could possibly serve as a reservoir of cancer viruses transmissible to man. Nearly as many different viruses are known to be transmitted from animals to man as are known to be indigenous to the human species. Viruses known to cause cancers have been shown under laboratory conditions to frequently induce infection and tumors in heterologous hosts. Although the likelihood of such events occurring in humans under natural conditions may not seem very great, the possibility that this may occur in some very intimate situations must still be considered.

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PROGRAM RESOURCES AND LOGISTICS

Drs. R. Holdenried and H. G. Steinman

The objective of the Program Resources and Logistics Section is to provide resources and logistical support primarily to the Special Virus-Leukemia Program and secondarily to other cancer or virus research projects. The resources consist of cell cultures, special laboratory animals, tumor and laboratory animal virus reagents, and normal and malignant human tissues as needed. The Section staff provides special technical guidance and assist in the management of projects in other areas of the Program. Dr. Kinard acted in this capacity on the major primate contracts. A testing service for murine virus antibodies and antigens is also provided.

Under contract support, academic, nonprofit or commercial organizations develop and produce the resources for Section distribution. Distribution policy may vary according to the type and demand for each kind of material. Generally, SVLP requirements receive first priority with other cancer research receiving secondary consideration. Items available in quantities in excess of anticipated Program demand can be issued to other National Institutes of Health projects, both intramural and extramural, and on a restricted basis for use in non-NIH associated work.

All resources available through the Section are listed and described in an annually revised catalog. In addition to the catalog description, information maintained on each item includes origin, processing procedure, degree of purity (freedom from contamination), infectivity and/or titer or other biological activity and, in the case of human tissue, pertinent clinical data on the donor.

The resource program, at the beginning of FY68, was split from the Viral Carcinogenesis Branch and attached as a Section to the Office of the Associate Scientific Director for Viral Oncology to service primarily the activities under the supervision of this Office. The restricted budget available to Viral Oncology in FY68, together with the plans for expansion of research activities, stimulated a re-evaluation of existing resource projects for Program relevance. Those projects judged nonessential or not of immediate need were reduced or programmed for termination. As a result the human tissue procurement activities were greatly reduced and several other projects were terminated. Future projects will be primarily limited to current Viral Oncology program needs.

Cell Culture Reagents -- The cell culture program is designed to provide new lines of cell cultures for use in the Viral Oncology program, as well as reagents such as nutrient media required for their cultivation and utilization.

Cultures of cell lines are established from a variety of human and animal tissues. Animal sources are selected to provide a broad spectrum of potential virus susceptibilities and serve as the basis for comparative biologic studies (University of California, Contract PH43-63-13). Human source materials are selected to provide cell strains hitherto unavailable, such as normal cells in the diploid state (Wistar Institute, Contract PH43-62-157), and tissue cells considered unlikely to harbor latent oncogenic viruses (Marquette University, Contract PH43-64-52).

The original project to make standardized cell cultures available for research in viral oncology has succeeded to the point that direct support by this program is no longer required. These are available from the ATCC, which had on deposit at the end of 1966, 69 certified cell lines.

A repository of several hundred partially characterized cell lines have been developed under the SVLP (Microbiological Associates, Inc., Contract PH43-68-706). Species of origin include chicken, cow, dog, cat, pig, monkey, ferret, hamster, mouse, rat, rabbit and seal as well as man.

Methods have been developed for the large-scale propagation of human hematopoietic cell lines derived from the white blood cells obtained from normal donors and from patients with leukemia, lymphoma or other neoplasms (Health Research, Inc., Contract PH43-65-616). Virus-like particles are associated with many of the cell lines obtained from the leukemic patients.

A register of human leukemic cell lines is being compiled as an information resource for all scientists conducting research in the leukemias and lymphomas (Program Analysis & Communication Section). The Human Cell Line Registry will contain all the available information on growth characteristics, virus association and virus susceptibility, cytology, karyology and oncogenicity as well as clinical data on the patient of origin.

Human Tissue Resources -- During FY68 the following contract centers participated in the Human Tissue Procurement Program: Health Research, Inc. (PH43-63-43), City Hospital of Padua, Italy (PH43-66-892), and M.D. Anderson Hospital, University of Texas (PH43-66-937) procured human malignant tissue. Marquette University (PH43-64-927) procured human embryonic and perinatal tissue. Georgetown University (PH43-67-1134) procured normal adult tissue. Korle Bu Hospital, Accra, Ghana, University of Ibadan, Nigeria (PH43-67-674), and Makerere University, Kampala, Uganda (PH43-67-47) supplied materials from patients with Burkitt tumor. Hospital for Sick Children, Toronto, Canada (PH43-65-97) and Montreal Children's Hospital (PH43-65-1020) collected plasma and sera from cases of childhood leukemia. Melpar, Inc. (PH43-68-80) acted as a processing center for perinatal tissue.

The procurement centers obtained materials from a total of 1,333 cases during the calendar year 1967. This figure includes both autopsy and surgical specimens. Tissues procured amounted to 22,600 grams and sera totaled 16,880 vials, of which 20,920 grams of tissue and 361 vials of serum were distributed. This tissue and serum comprised a total of 2,715 specimens shipped. Regular shipments of well characterized human perinatal kidney suspensions have been made to selected recipients. This program has provided a dependable source of human kidney cells for virologic studies.

A large amount of clinical information related to the specimens is processed by means of a computer program which was completed in FY68, by the computer division of NIH. Weekly runs are now made upon the IBM 360 computer at NIH.

Studies on the viability of tissue preserved in liquid nitrogen and of fresh tumors, began at Medical Research Consultants, Inc. (PH43-67-1368). These studies are currently in progress and are elucidating the important parameters for tumor cell growth from freshly obtained human tumors. The type of cellular outgrowth and the effects of various substrates and media on it are being studied. Cooperative studies between Medical Research Consultants and the United States Navy Tissue Bank are being undertaken to find better ways to preserve tissues and cell cultures by freezing. Under contract at Massachusetts General Hospital (PH43-65-1034) methods for preserving peripheral human leukocytes are underway. Methods have been developed in 1967 for the collection and preservation of human lymphocytes with high recovery of viability. Attempts to preserve human granulocytes are currently underway. Preliminary reports are promising. Methods for the collection and separation of platelets have also been developed.

During FY68 it was determined by advisory committees of SVLP that the Cancer Institute would discontinue its tissue procurement for nonprogrammed cancer research. Consequently, it was determined the following contracts would be discontinued because their relationship to specific program objectives was not as great as the remaining contracts:

Health Research, Inc.
Baltimore City Hospitals
Georgetown University
Hektoen Institute
Massachusetts General Hospital
Medical Research Consultants
Melpar, Inc.
Tulane University

As a result of this decision, in the FY69 only tissues that are specifically requested by program components will be made available.

Laboratory Animal Program -- The general objective of the resource program in the area of laboratory animals is to define those animals of particular usefulness in viral oncology studies with emphasis on improved quality and breeding efficiency and then promote their use until constant and economical animal resources of high quality are available. Program needs and relatively good opportunities for success have continued to confine these efforts to two broad areas: (1) breeding and holding of primates, and (2) virus-defined rodents and chickens.

Major portions of the SVLP involve the inoculation of newborn primates with human cancer material and candidate viruses. Rhesus monkeys and baboons, while widely used, are expensive to keep for the long observation periods required for cancer studies and they may not be the most susceptible primate species for viral oncogenesis. Therefore, the breeding potential of small, relatively less expensive, primate species is being investigated.

A small primative primate, Tupaia glis, the tree shrew from Thailand, of intermediate relationship to rodents and the more highly developed primates, would be specially valuable if susceptible to the viruses of both rodents and primates. Under Contract PH43-64-801 a moderately successful breeding colony of tree shrews has been established. While it is not yet producing enough young for experimental use, limited study of its viral susceptibility has been initiated. One animal with a spontaneous sarcoma was sent in January 1968 to Baylor University where, under Contract PH43-68-678, the susceptibility of cell cultures from this sarcoma to a variety of viruses is being tested. Test results are not yet available.

The San Diego Zoo project, Contract PH43-63-56, studies the breeding potential of small primates. Young from those species reproducing adequately are channeled to various research projects to determine their usefulness and to stimulate the development of other breeding colonies. With the establishment of breeding colonies elsewhere the continuation of that particular species at the San Diego Zoo project becomes less critical and attention can be directed to other species. The bushbaby, Galago crassicaudatus, a prosimian of guinea pig size, breeds well producing 2 to 3 young per female per year. It has been used in thalinamoid and chemical carcinogen studies and is scheduled for viral experiments. A colony of Miopithecus talapoin, a member of the African green monkey complex of species but less than one-half the size of the rhesus, has recently been started. After a couple of years of exhaustive effort to purchase breeding stock of talapoins through dealers failed, the Project Director went to West Africa and obtained the first adults directly for the colony. Steps for the development of a continued supply through a commercial channel were also taken.

The other major effort of the animal resources program has been to try to eliminate some of the confusion and loss caused by indigenous rodent and chicken viruses from animals used in research. Under Contract PH43-67-700, Microbiological Associates, Inc. continues a long term virus study and murine virus diagnostic service. The contract, under the careful guidance of the Project Officers, has developed a capability of determining the significance of indigenous murine viruses in cancer research test systems. Investigation of lymphocytic choriomeningitis infections in mice and tumor inoculums in the year resulted in the development of more sensitive diagnostic tests and the uncovering of a previously incompletely recognized problem. Under a separate, non-NCI contract NASA utilizes information and investigative techniques made available by this project.

The search for a leukemia virus-free mouse by Lobund under Contract PH43-65-1018 terminated with substantial evidence that all laboratory mouse strains, even in "germfree" status, are infected with Type-C particles. "Germfree" rats, however, at least of Lobund stock, do not appear to carry leukemia virus.

A nucleus flock of disease free chickens was maintained for another year by the University of Connecticut under partial support of Contract PH43-62-505. Marek's disease appears to be the most difficult infection to exclude.

Viral Reagents Production -- The Program Resources and Logistics Section governs the production, storage and distribution of oncogenic viruses and virus reagents, and provides a description (testing and monitoring) of the virus products for the research community. The virus reagents in the Program have been developed and produced for use in the identification and characterization of cancer-virus antigens in animal species and humans.

A number of laboratories produced and, in one case, distributed the following RNA oncogenic viruses: (1) the murine leukemia viruses--Rauscher virus harvested from mouse plasma and spleen (Hazleton Laboratories, Inc., Contract PH43-65-635), Moloney virus harvested from mouse plasma and spleen (Melpar, Inc., Contract PH43-65-1033), and Germfree Products, Inc., Contract PH43-64-533), and Gross, Friend, Rich, Breyere-Moloney and Manaker viruses (Germfree Products, Inc., Contract PH43-64-533), and (2) the sarcoma viruses --Moloney sarcoma virus and pseudotypes, Rous sarcoma and antisera pseudotype viruses (University Laboratories, Inc., Contract PH43-66-1133). Of all the viruses mentioned, the only contracts now active under the Program Resources and Logistics Section are with Hazelton Laboratories (currently to produce Rauscher leukemia virus) and with University Laboratories (to produce Moloney sarcoma virus and antisera, Moloney pseudotype viruses and Rous sarcoma). These are presently the cancer viruses in greatest demand.

Moloney, Rauscher, and other animal leukemia viruses are also produced by laboratories outside of the Program Resources and Logistics Section, but they are, for the most part, included in the storage and distribution phases of the program.

Known viruses associated with various animals are needed by investigators who lack the time, funds, or background to obtain these reagents for themselves. The availability of uniform and certified reference materials is of greatest importance to the whole Etiology Area.

Thus far, the Program distributes or has in production "working" or diagnostic reagents for feline, canine, and simian viruses, as well as the murine leukemia viruses and sarcoma viruses (Cornell University, Contract PH43-65-664 and Dow Chemical Company, Contract PH43-67-657). Active projects include the preparation of immune sera to dog and cat viruses in goats, to develop high titer antisera for murine leukemia viruses (Baltimore Biological Laboratories, Contract PH43-63-1161), and to prepare and test reference reagents (seed material and antisera) for simian foamy viruses Types 1-7 (University of Louisville, Contract PH43-66-902) and other simian viruses (Southwest Foundation for Research and Education, Contract PH43-66-950). The plan, however, is to gradually phase out the reagent production contracts. The reagent, as received, will be packaged by Ben Venue Laboratories, Contract PH43-68-633.

The main problem in the whole virus production program has been intermittent contamination of viral products either at the onset of production via contaminated animals or virus seed stock, or during the course of production via outside contamination. Viruses such as LDH, Sendai, Reo 3, lymphocytic choriomeningitis virus, minute virus of mice, and mouse hepatitis virus have been the most common contaminants of the RNA oncogenic viruses, while mycoplasma has been the most common contaminant of the non-murine viral reagents. The presence of LCM and hepatitis viruses or mycoplasma in viral products has made them of marginal use for cancer research. Contamination is determined by standard tests performed under contracts with Microbiological Associates, Inc. (PH43-67-700) and the Wistar Institute (PH43-65-1002). when contamination is reported, concerted effort on the part of animal supplier, contractor, and Resources and Logistics Section is brought forth to determine and eliminate the source of the problem.

The Program Resources and Logistics Section fulfills the needs of the general research community on a priority basis--NCI, NIH, academic institutions, and commercial laboratories, in that order. A fluctuating research demand in quantity and also kind of virus reagents introduces supply problems detected largely by the Resources and Logistics Section by changing patterns of distribution of the various virus products issued by the Repository. Reagent production reflects issue experience and guidance by the SVLP for anticipated projects.

BIOHAZARDS CONTROL AND CONTAINMENT

Dr. A. Hellman & Mr. E. Barkley

This section evaluates and performs research in virology, biochemistry and bioengineering in order to refine and develop new environmental control techniques. A necessary adjunct to this effort is the development of techniques for the rapid detection of hazardous conditions in the laboratory and determining environmental parameters that might enhance host susceptibility to infection.

This section serves as a biomedical and bioengineering resource to the Viral Oncology program and NCI in general. Since many of the research contracts of other portions of the SVLP and other areas of the NCI involve research with oncogenic viruses, problems pertaining to containment or safe handling of such materials arise. Where feasible, this Section then provides expert consultive advice, within limits of available information, to many universities, non-profit corporations, or commercial firms under contract to NCI. Such consultation activities involve some or all of the following components: (a) Review of the scope of activity to identify specific problem areas; (b) Development with contractor personnel of a work scope or program of requirements for renovation or new construction projects; (c) Participation in site visits to contractors' facilities to evaluate existing containment equipment and techniques; (d) Review of construction drawings for proposed facilities at various stages during the design; (e) Assistance or recommendations to improve selection of containment equipment; (f) Suggest medical monitoring procedures for laboratory personnel.

3. PROGRAM MANAGEMENT ACTIVITIES (SVLP)

a. Summary

From the research standpoint the Special Virus-Leukemia Program is divided into 4 major areas of effort: Human Leukemia Etiology and Prevention, Special Animal Leukemia Ecology Studies, Biohazards Control and Containment, and Human Leukemia Therapy. Operationally the Program has been divided into 7 program segments -- Developmental Research, Testing and Monitoring, Resources and Logistics, Epidemiology, Special Animal Leukemia Ecology Studies, Human Leukemia Therapy, and Biohazards Control and Containment. These working groups, which with the exception of the Human Leukemia Therapy Segment, are chaired by senior scientists (see below) within the Etiology Area, who are responsible for the development of research programs in their respective segments within the context of the overall plan. An eighth group, the Solid Tumor-Virus Segment, has been identified, the activities of which began in August 1967.

The Program Segment Chairmen, together with their Project Officers and other key staff scientists provide the inhouse intellectual and technical motivation, guidance and thrust for the Program. Scientific activities are being conducted principally within the Etiology Area and the Chemotherapy Area of NCI, and through 105 contract laboratories. Each contract is continuously monitored by repeated personal contacts between the NCI Project Officer and the contractor, and through an effective analysis and communications system including preparation by the contractor of triannual progress reports. Key modifications in workscope and/or project emphasis are made according to the performance of the contractor, and, most importantly, according to the precipitous or predictable changing needs of the Program, the needs and changes of which are predicated both on excellence and relevance to the expeditious attainment of the objectives of the Special Virus-Leukemia Program.

PROGRAM SEGMENT	CHAIRMEN & VICE CHAIRMEN (FY 67)	CHAIRMEN & VICE CHAIRMEN (FY 68)
DEVELOPMENTAL RESEARCH	Dr. Robert Manaker Dr. Albert J. Dalton	Dr. Robert Manaker Dr. Albert J. Dalton
TESTING & MONITORING	Dr. Robert Stevenson Dr. Mary Fink	Dr. Mary Fink Dr. James Duff
EPIDEMIOLOGY	Dr. Robert Miller	Dr. Robert Miller
RESOURCES & LOGISTICS	Dr. Robert Stevenson	Dr. Robert Holdenried Dr. Harry Steinman
BIOHAZARDS CONTROL & CONTAINMENT	Dr. William Payne Mr. Robert Runkle	Dr. Alfred Hellman Mr. Emmett Barkley

SPECIAL ANIMAL LEUKEMIA ECOLOGY STUDIES	Dr. John Moloney Dr. Robert Holdenried	Dr. John Moloney Dr. Michael Chirigos
HUMAN LEUKEMIA THERAPY	Dr. Seymour Perry Dr. Edward Henderson	Dr. Seymour Perry Dr. Edward Henderson
SOLID TUMOR-VIRUS	--	Dr. Robert Huebner Dr. James Duff

Principal Activities of the Joint Chairman -- The chairmen have two main operational functions: One with respect to the responsibilities involved in chairing the various working groups, and another with respect to the responsibilities involved when all 8 chairmen meet in joint session.

The initial and probably the most important function is concerned with the planning of the scientific projects to be accomplished in each working segment for the oncoming year. This is a critical function because it is performed on the basis of an anlaysis of the results of ongoing efforts not only in the respective area but also in all other program areas as they may pertain to the particular work of a program segment. It is on the basis of these initial plans that more specific work statements of the research to be performed are prepared, and upon which contractors are invited to submit specific proposals for the performance of the work. At this stage working group members and other invited experts are responsible for the conduct of site visits to potential contractors' facilities as required, and to assess the competence of the contractor to perform the desired work. In those situations where more than one contractor has been invited to submit proposals for the same research effort, then it is the responsibility of the working group members to select from several proposals that proposal and that contractor which in their opinion is most qualified to perform the work. The recommendation of the working groups as to contractor selection are then forwarded to the Joint Chairmen and to the Scientific Directorate for final review and approval. In his capacity as Chairman of the working group, the Segment Chairman, in addition to the usual responsibilities associated with the effective functioning of advisory groups, is also responsible for bringing to the working group sessions all information relevant to the deliberations and decisions of the working groups not only from the ongoing activities of the SVLP, but also from any other program areas of the Institute which may be relevant.

The Joint Chairmen's meetings represent the major vehicle through which the chairmen perform their responsibilities as the focal point of total program operations within the Institute and for which they are directly responsible to the Associate Scientific Director for Viral Oncology, the Scientific Director for Etiology and the Director of the Institute. The main function of these joint sessions is to provide the basis whereby a synthesis and integration of the activities of each segment is achieved with respect to the differential impact of these achievements on total program objectives. It is at the joint sessions that the relative priorities of research established in each working area are reviewed with respect to establishing an overall priority for the total program. This is an extremely difficult but important step because the items considered to be of high priority within the perspective of an individual program segment do not necessarily achieve the same level of priority within the perspective of total program. Thus, in joint session, the chairmen simultaneously or in sequence perform the review, analytical, integrating, developmental, and program decision activities required for the effective operation of the program.

Beginning in June 1967, a Branch and Associate Branch Chiefs group was formed composed of Dr. Rauscher, Chairman, Drs. Moloney and O'Connor, VLLB, Drs. Dalton and Manaker, VBB, Drs. Huebner and Duff, VCB, Dr. Bryan, Science Coordinator in Viral Oncology, Mr. Gerald Meyer or his designate, and Dr. Reisinger as Assistant for Laboratories, for in depth consideration of SVLP and Branch problem areas and for further guidance and planning of the overall program. The group meets on the first Wednesday of each month at 2:00 p.m., in the Wiscon Building, Room 502. The principal activities of the group will be to advise the chairman (ASD) on major scientific and administrative issues; to exchange and assess problems and progress at the project and program levels of SVLP and STVP (Solid Tumor-Virus Program); and to serve as an ad hoc group for the primary review of contracts. The latter activity will be conducted infrequently and only when it is not possible to schedule an adequate and timely review by existing working groups. On these few occasions it will probably be desirable to supplement the group with other NIH or perhaps outside investigators.

Program Analysis and Communications (PAC) -- The PAC Section serves primarily as an information center in the SVLP for purposes of program management, but also functions as the plexus for scientific communication within the Program. Its responsibilities require both the statistical approach and the exercise of techniques of information collection, summarization, and dissemination. Since the Special Virus-Leukemia Program is essentially a nation-wide network of coordinated research, involving the cooperative efforts of scientists at the NIH, with other scientists located in numerous institutions throughout the United States, an active communications system embracing more than the conventional aspects of data retrieval, analysis, and dissemination is essential.

Data retrieval and statistical operations currently underway in PAC are: (1) Correlation of results of laboratory virus detection with clinical characteristics of specimen donors for patients hospitalized with leukemia and lymphoma at the NIH Clinical Center and at the M.D. Anderson Hospital and Tumor Institute; (2) Management and analysis of findings of the Comparative Serology Survey (a comparison of methodologies in the immunological approach to detection of viral antibodies; project initiated by Dr. Mary Fink, NCI), (3) Establishment of a comprehensive international register of existing human lymphoid cell lines; (4) Maintenance of a computerized and updated inventory of leukemia specimen aliquots processed by the Laboratory of Viral Oncology, NCI, and by the M.D. Anderson Hospital; (5) Management of the system of progress reports from participating program contractors.

The Information Unit of PAC assumes full responsibility as an information resource for all publications (past and current) and current research projects in the field of viral oncology. Services are: (1) Viral Tumorigenesis Report: preparation and distribution semiannually of summaries of current research projects in viral oncology; (2) Reading Guide to the Cancer-Virology Literature: a monthly publication featuring abstracts of papers in the field of viral oncology, from scientific journals throughout the world; (3) Bibliographic Service: a new, rapid, library service which will supply lists of reference citations of published literature in viral oncology upon request, and with specific reference to the parameters of the search requested; searching service is offered to all SVLP related scientists whether in or outside of NIH.

Intersegment Coordination -- The Office of the Scientific Coordinator in Viral Oncology (Dr. W. Ray Bryan) was established within the Office of the Associate Scientific Director for Viral Oncology in 1967 to promote the more efficient coordination within that Office of collaborative research in certain broad, critical, areas of major program emphasis requiring the mobilization of scientific skills and specialties across administrative and program-management organizational boundaries. The largest of the three separately identified viral oncology programs, the Special Virus-Leukemia Program (SVLP), is made up of eight working segments led by intramural scientists drawn from each of the three Branches of the Office of the Associate Scientific Director for Viral Oncology, as well as from another subdivision of NCI, Chemotherapy. A second program, involving viral oncological aspects of the Breast Cancer Task Force activities, also is collaborative with another subdivision of the NCI, namely General Laboratories and Clinics. Although the overall coordination of joint activities which cut across these major boundaries is at the level of the Associate Scientific Director for Viral Oncology, operational coordination of certain of the broad multifaceted research undertakings in these programs is delegated to the Scientific Coordinator in Viral Oncology.

In order to expedite the application of new principles (detailed in other sections of this report) to the human problem, as well as to gain new knowledge that might speed the attainment of this objective, a concerted collaborative research effort on the C-type oncogenic viruses was organized among scientists of groups within the SVLP who were already committed to the study of these agents. No new funding was involved, but some of the supporting activities of several key contractors were reoriented to include support for these new integrated activities. Significant collaborations between investigative groups were already in progress, but broader coalitions of skills, disciplines and special facilities were needed for embracing certain problems that needed to be worked upon, as well as for development of the special virus-host systems that are required for some of them. Of prime importance was the bringing together of the groups responsible for the development and production of resource materials and those who would use them in immediate research pursuits, so that the latter groups could monitor them from the start and determine their suitability for the uses intended. This was in contrast to the former practice of having a separate advisory group on resources per se, attempt to project and provide for the needs in specific research areas. The inefficiency of the latter procedure was pointed up by the Resources and Logistics Advisory Working Group itself, which requested that its responsibilities be restricted to the more general program needs of the SVLP and that the more specific resources for particular activities within highly specialized areas be dealt with through some other mechanism. The Associate Scientific Director for Viral Oncology transferred the responsibility for such special resources and services to Program Management within his Office, and designated as the reviewing body the senior intramural staff of Viral Oncology -- Branch and Associate Branch Chiefs, Scientific Coordinator, Assistant for Laboratories (Executive Secretary), and Associate Scientific Director (Chairman).

Proceeding under dual review by this group and by the Joint Chairmen body made up of SVLP Segment Chairmen (of which the Associate Scientific Director is also general chairman), progress has been made in the development of two types of collaborative program activities: (1) The activation of joint research projects for which all special resources and facilities were available for immediate application; and (2) The initiation of actions to develop systems and resource materials which are now lacking, but which are needed for high priority studies to be launched as soon as they become available.

Largely within the past two years there developed a need to coordinate other like scientific activities conducted by several Program Segments. As an example, electron microscopy is a part of five different contracts within the Developmental Research Segment and is being coordinated within the Segment by the Program Segment Chairman. Similarly, electron microscopy studies within the Special Animal Leukemia Ecology Program Segment are being coordinated by the Chairman of this Segment. The use of similar instruments, techniques, and approaches as well as the occurrence of problems common to both Segments necessitated intersegment planning, implementation and information exchange. Accordingly, the following people accepted responsibility for coordinating scientific activities of importance to the overall program. While these responsibilities have somewhat low visibility, they are no less important to the SVLP than that related to Segment Chairmanships within the Program.

(1) Dr. A. J. Dalton has assumed responsibility for coordinating detection, monitoring and other studies conducted principally through electron microscopy. This informal group is composed of all electron microscopists within the Program and is charged with the development of definitive criteria for the morphologic identification, characterization and quantitation of virus particles; the development, test and use of optimal microscopy techniques, and the identification of artifacts (PPLO, normal cellular constituents, etc.) and technical deterrents to the effective application of electron microscopy within the SVLP.

(2) Dr. Mary Fink has assumed responsibility for coordinating the immunological and serological testing activities related principally to studies with known human viruses and with candidate viruses recently isolated from human leukemia and lymphoma materials. She and Dr. James Duff, VCB, Etiology, NCI, are attempting to provide adequate quantities and kinds of standardized antisera for comparative testing throughout the Program. Most recently she and an ad hoc working group composed of both NIH and contract investigators have developed a project to determine which of 14 different serological tests will reliably and economically detect antibody in large numbers of human serums. These sera will be tested for antibodies to several isolates of the herpes-type virus present predominantly in cell lines established

from human leukemia and lymphoma patients. The results of this study may very well provide the means for conducting large scale sero-epidemiological surveys aimed at establishing, at least indirectly, a causative relationship between candidate human viruses and human leukemia and lymphoma.

(3) Dr. Timothy E. O'Connor has assumed responsibility for planning, implementing, and coordinating both inhouse and contract activities concerned with the biochemical and biophysical concentration, purification, and characterization of known animal leukemia viruses and of candidate viruses recovered from man. This responsibility includes the training of contract investigators such as Dr. Charles Burger, Upstate Medical Center, Syracuse, New York, Mr. Irvin Toplin, Pfizer Laboratories, Maywood, New Jersey, and others in the use and application of density gradient centrifugation, electrophoresis and chromatographic column separation techniques. Inasmuch as viruses recovered from human leukemia and lymphoma patients have to date not shown biologic activity, it is of continuing paramount importance that comparative studies be conducted to determine whether candidate human viruses have biochemical and biophysical characteristics in common with infectious animal and human viruses.

The directive to and the responsibility of the National Cancer Institute to plan, implement and effectively manage national and international programs for the control of neoplastic diseases make it mandatory to attract, train and retain a hard core of effective program managers. Such people must have the unusual blend of scientific experience and excellence as well as willingness, desire and skill to coordinate people and activities toward the attainment of specific program objectives. The effective overall management of the Special Virus-Leukemia Program since its inception and particularly during FY 68 is due nearly entirely to the unselfish perseverance of the Science Coordinator in Viral Oncology, the Program Segment Chairmen and Vice Chairmen, their associates including Project Officers, to the leaders of the informal ad hoc intersegment groups indicated above and, substantially, to the motivation and daily drive of the Administrative Officer for Etiology, Mr. Gerald Meyer, and his associates including Mr. Richard Miller, and Mr. Thomas Lewin and Mr. Fred Shaw, the latter of whom have responsibility for the fiscal management of all SVLP contracts in the Etiology Area. The consistent excellence of the Secretary to the Chairman, Mrs. LaVerne Stacy, and her associates have had a measurable beneficial impact on overall program management.

b. Working Groups and Members

General -- The program segment working groups are the basic operational units for the Special Program. They are composed of both Federal and non-Federal scientists representing expertise in the various areas of research involved in the program. Each working group is chaired by a senior NCI staff scientist. Although the primary function of the working groups is to provide advice to the Institute on the research and scientific aspects of the program, the participation of the members of the working groups in a variety of program operations is more extensive than the typical advisory committee. The members help and advise the respective chairmen in the development of program plans for their specific area of research for each fiscal year; develop work statements for specific research contracts; make site visits to contractor facilities; review contractor proposals and when competitive proposals are received select the best qualified contractor for recommendation to the Scientific Directorate; and participate in the very important functions of analysis and evaluation of the results of ongoing research accomplished in each of the eight segment areas -- this analysis and evaluation provides the basis for many major program decisions.

Appointment of Members and Tenure -- Both Federal and non-Federal members are invited (by letter) to serve on the working groups by the Director, NCI, with advice from the Scientific Director for Etiology and the respective Segment Chairmen. The letters of invitation are prepared by the Chairmen for the Director's signature. Members are selected solely on the basis of their specific expertise and willingness to serve. There are no geographical or other considerations. Non-Federal nominees who accept the invitation serve a four-year term on the working groups and are appointed as NCI consultants with the consultancy renewed each year for the length of tenure. However, since the Special Virus-Leukemia Program is new, the length of tenure for some members of the initial working groups will vary. No individual can serve concurrently on more than one working group and, upon serving the full term on one group, no individual can be appointed to another working group until at least one year has intervened. All members are voting members with respect to the deliberations and recommendations of the working groups. There are no ex officio voting members on the working groups. However, as program requirements dictate, a chairman may appoint special ad hoc groups to advise him on particular aspects of the program in which expertise is not represented among the regular working group members. Tenure for non-Federal members cannot be extended beyond the four year term except under special circumstances and conditions, and then only with the specific approval of both the Scientific Director for Etiology and the Director, NCI.

The Segment Chairmen and other NCI staff members will serve on the working groups for the full duration of the program, except as they are given other priority assignments by the Scientific Director for Etiology, or as they specifically request to be released from this responsibility. In general, the tenure of Federal members, other than NCI staff, will be for four years, but may be extended beyond this dependent upon current program requirements and staffing situations.

Rotation of Members -- All non-Federal members will be rotated on a staggered basis so that one quarter of the membership will be rotated each year beginning in the fourth year of the program. Since the staggered rotation cycle must be initiated, some members of the initial working groups will serve only three years, while others will serve seven years. These arrangememts will be made by mutual agreement between the individual member and the respective Segment Chairmen.

Rotation of all Federal members who are not chairmen or members of NCI staff, will generally take place on a four-year staggered basis unless program requirements indicate the desirability of extending beyond the four-year term the membership of certain Federal scientists on the working groups.

Preventing Conflicts of Interest -- In general, non-Government members of the working groups are expected to participate in all working group functions. However, in some cases special problems may arise concerning the existence of a conflict of interest situation.

For the purposes of this program, a conflict of interest is felt to exist if either of the following two conditions apply:

1. The non-Government member is an employee of or a consultant to one of the potential contractors being considered for contract award; or

2. The non-Government member is an employee of or a consultant to a profit-making organization which is not being considered as a potential contractor for a particular project but which is in general business competition with one of the organizations being considered for contract award.

Whenever one of these conditions is established with respect to a non-Government member of a working group, then his participation in the activities related to the development of the specific contract is limited to program planning functions and the development of the contract work statement. He cannot participate in site visits to contractor facilities where potential conflict of interest exists, in development of proposals with potential contractors, nor in the preliminary review of proposals for contractor selection.

Under such conditions, the program segment chairman, at his discretion, is responsible for augmenting the membership of the group with ad hoc advisors to assure adequate technical review of the proposed contracts.

WORKING GROUP MEMBERS, SVLP, ETIOLOGY AREA, NCI

DEVELOPMENTAL RESEARCH

ORIGINAL		1967 - 1968	
W. R. Bryan	NCI, NIH	R. A. Manaker	NCI, NIH
A. J. Dalton	NCI, NIH	A. J. Dalton	NCI, NIH
R. Manaker	NCI, NIH	T. O'Connor	NCI, NIH
T. O'Connor	NCI, NIH	A. Rabson	NCI, NIH
A. Rabson	NCI, NIH	S. Stewart	NCI, NIH
S. Stewart	NCI, NIH	P. Gerber	DBS, NIH
P. Gerber	DBS, NIH	J. Hartley	NIAID, NIH
J. Grace	Roswell Park	W. Henle	Children's Hosp. Philadelphia
W. Henle	Children's Hosp. Philadelphia	G. Foley	CCRF, Boston
A. Howatson	U. of Toronto	A. Howatson	U. of Toronto
A. Moore	Sloan-Kettering	B. Roizman	U. of Chicago
		D. Walker	U. of Wisconsin
		L. Old	Sloan-Kettering

TESTING AND MONITORING

ORIGINAL		1967 - 1968	
R. Stevenson	NCI, NIH	M. Fink	NCI, NIH
M. Fink	NCI, NIH	J. Duff	NCI, NIH
A. Dalton	NCI, NIH	A. Dalton	NCI, NIH
R. Malmgren	NCI, NIH	R. Malmgren	NCI, NIH
C. Stark	NCI, NIH	J. Sever	NINDB, NIH
J. Sever	NINDB, NIH	T. Borsos	NCI, NIH
R. Couch	Baylor U.	R. Couch	Baylor U.
L. Hayflick	Wistar Inst.	L. Hayflick	Wistar Inst.
		A. Brown	Ft. Detrick
		N. Schmidt	Calif. PHS Labs.

WORKING GROUP MEMBERS, SVLP, ETIOLOGY AREA, NCI

PROGRAM RESOURCES & LOGISTICS

ORIGINAL		1967 - 1968	
R. Stevenson	NCI, NIH	R. Holdenried	NCI, NIH
V. Evans	NCI, NIH	H. Steinman	NCI, NIH
W. Heston	NCI, NIH	V. Evans	NCI, NIH
D. Axelrod	NIAID, NIH	W. Rowe	NIAID, NIH
W. Rowe	NIAID, NIH	A. Webb	NIAID, NIH
A. Webb	NIAID, NIH	R. Yoder	Tulane Univ.
H. Morgan	U. of Rochester	R. Atchison	PH, U. of Pittsburgh
R. Yoder	Tulane U.	T. Berge	ATCC
		L. Neurauter	Univ. of Ga.
		K. Sell	NMRI

EPIDEMIOLOGY

ORIGINAL		1967 - 1968
R. Miller	NCI, NIH	
B. MacMahon	Harvard U.	
N. Mantel	NCI, NIH	No change
R. Seltser	Johns Hopkins	
R. Tjalma	NCI, Michigan	

WORKING GROUP MEMBERS, SVLP, ETIOLOGY AREA, NCI

SPECIAL ANIMAL LEUKEMIA ECOLOGY

ORIGINAL		1967 - 1968	
J. Moloney	NCI, NIH	J. Moloney	NCI, NIH
R. Holdenried	NCI, NIH	M. Chirigos	NCI, NIH
J. Glynn	NCI, NIH	G. Burton	NCI, NIH
M. Stanton	NCI, NIH	J. Glynn	NCI, NIH
B. Eddy	DBS, NIH	M. Stanton	NCI, NIH
R. Dougherty	State U. of N. Y.	B. Eddy	DBS, NIH
V. Groupé	Rutgers U.	R. Dougherty	State U. of N. Y.
W. Moloney	Boston City Hosp.	V. Groupé	Rutgers U.
C. Rickard	N.Y. Vet. College	C. Rickard	N.Y. Vet. College
G. Theilen	U. of Calif.	F. Deinhardt	Presb.-St. Luke's
J. Trentin	Baylor U.	J. Grace	Roswell Park
		D. Pinkel	St. Jude's, Memphis
		C. York	Zoo. Soc., San Diego

BIOHAZARDS & CONTAINMENT

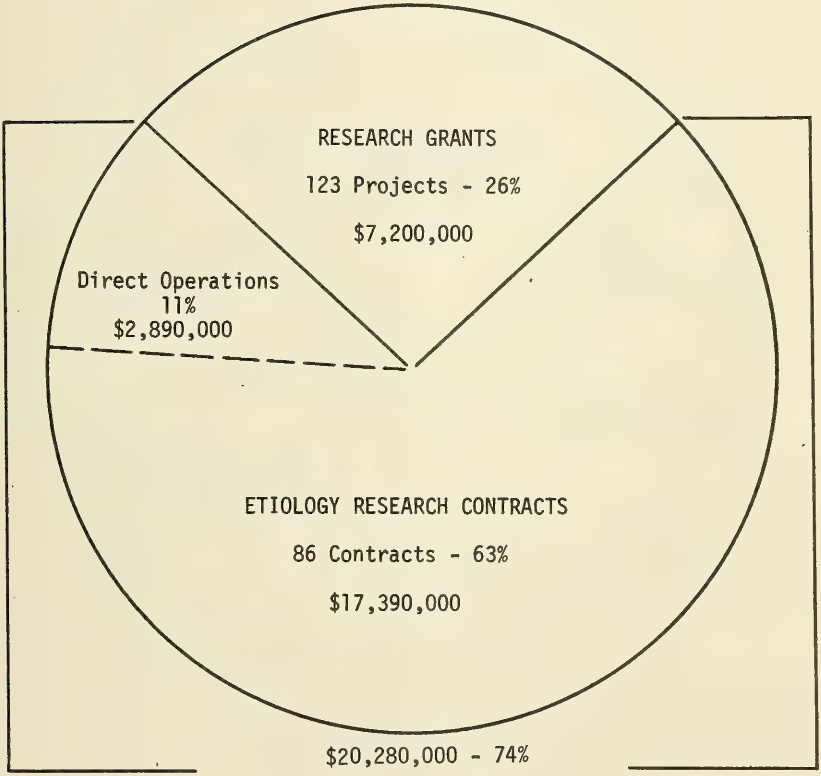
ORIGINAL		1967 - 1968	
W. Payne	NCI, NIH	A. Hellman	NCI, NIH
R. Runkle	NCI, NIH	E. Barkley	NCI, NIH
E. Barkley	NCI, U. of Minn.	W. Payne	DEHS, N. Carolina
J. Gainer	NCI, NIH	R. Bond	U. of Minnesota
A. Hellman	NCI, Oak Ridge	M. Chatigny	Naval Bio. Lab.
W. Penland	NCI, NIH	S. Madin	U. of Calif. (NBL)
R. Bond	U. of Minnesota	M. Mufson	U. of Illinois
S. Madin	U. of Calif.	A. Wedum	Ft. Detrick
M. Mufson	U. of Illinois	G. Phillips	Becton Dickenson
G. Phillips	Ft. Detrick	P. Gerone	Ft. Detrick
A. Wedum	Ft. Detrick		

WORKING GROUP MEMBERS, SVLP, ETIOLOGY, NCI

ORIGINAL		1967 - 1968	
HUMAN LEUKEMIA THERAPY			
G. Zubrod	NCI, NIH	S. Perry	NCI, NIH
A. Serpick	NCI, NIH	E. Henderson	NCI, NIH
S. Perry	NCI, NIH	D. Rall	NCI, NIH
E. Frei	NCI, NIH	S. Schepartz	NCI, NIH
D. Rall	NCI, NIH	A. Serpick	NCI, Baltimore
S. Schepartz	NCI, NIH	E. Frei	M. D. Anderson
J. Burchenal	Sloan-Kettering	J. Burchenal	Sloan-Kettering
J. Holland	Roswell Park	J. Holland	Roswell Park
S. Farber	CCRF, Boston	S. Farber	CCRF, Boston
		SOLID TUMOR-VIRUS (New Group)	
None		R. J. Huebner	NIAID, NIH
		J. T. Duff	NCI, NIH
		C. Boone	NCI, NIH
		H. Rapp	NCI, NIH
		W. Rowe	NIAID, NIH
		J. Melnick	Baylor U.
		A. Sabin	U. Cincinnati
		R. Dulbecco	Salk Inst.
		E. Lennette	Calif. PH
		M. Green	St. Louis Univ.

c. Funding Analyses by Major Areas

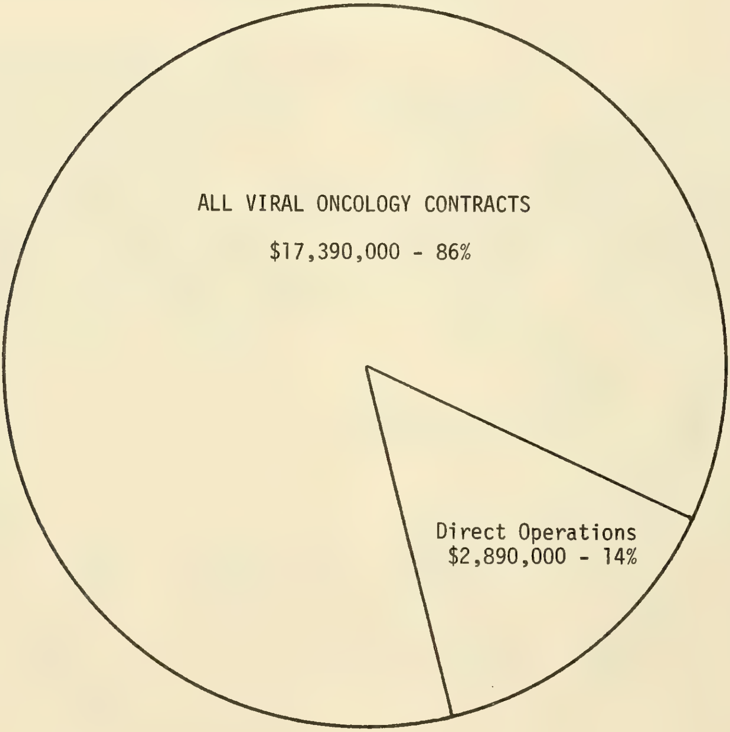
VIRAL ONCOLOGY RESEARCH BY
MAJOR SUPPORT MECHANISM
FY 1968 (Estimated)
NATIONAL CANCER INSTITUTE



VIRAL ONCOLOGY
ETIOLOGY AREA - NCI

FUNDING OBLIGATIONS - CONTRACTS AND DIRECT PROGRAM

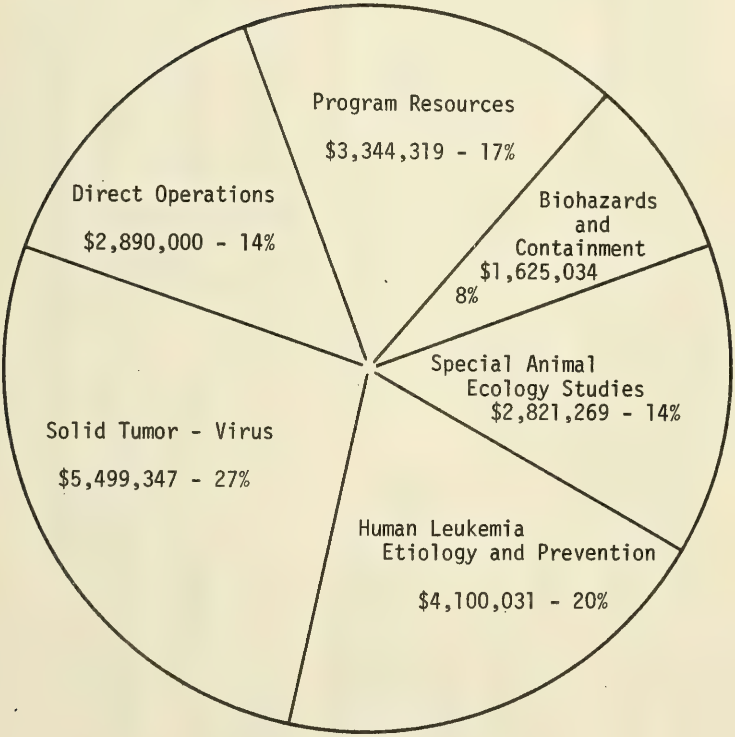
FISCAL YEAR 1968



VIRAL ONCOLOGY
ETIOLOGY AREA - NCI

FUNDING OBLIGATIONS BY MAJOR AREA

FISCAL YEAR 1968 - $20,280,000



COST ANALYSIS OF CONTRACT AND DIRECT ACTIVITIES IN THE VIRAL ONCOLOGY PROGRAM, ETIOLOGY AREA, NCI FY 1968 (Estimated)

	TYPE OF ACTIVITY AND PROGRAM AREA	NUMBER OF CONTRACTS [1/]	FY 1968 LEVEL		PERCENT	
I.	PROGRAM MANAGEMENT					
	A. Direct Operations			$2,890,000		14%
II.	HUMAN LEUK. ETIOLOGY & PREVENTION			4,100,031		20%
	A. Etiology (Objective 1)	28	$3,575,523		17.6%	
	B. Prevention (Objective 2)	5	524,508		2.5%	
III.	SPECIAL ANIMAL ECOLOGY			2,821,269		14%
	A. Etiology	21	2,684,961		13.2%	
	B. Prevention	4	136,308		.7%	
IV.	SOLID TUMOR-VIRUS			5,499,347		27%
	A. Etiology	20	5,326,814		26.2%	
	B. Prevention	3	172,533		.9%	
V.	PROGRAM RESOURCES & LOGISTICS			3,344,319		17%
	A. Human	22	1,512,706		7.5%	
	B. Animal	19	1,831,613		9.0%	
VI.	BIOHAZARDS CONTROL & CONTAINMENT			1,625,034		8%
	A. NIH Construction and Equipment	1	1,132,419		5.6%	
	B. Other Activities	3	492,615		2.4%	
	TOTAL	126		20,280,000		

[1/] Since many contracts include more than one activity, the totals of I-VI do not correspond with the total number of contracts. Actual number of contracts is 102.

NUMBER OF CONTRACTS BY TYPE OF INSTITUTION

VIRAL ONCOLOGY, ETIOLOGY AREA, NCI

	Profit		Educational		Non-Profit		Other		Total	
	No.	Amount	No.	Amount	No.	Amount	No.	Amount	No.	Amount
Human Leukemia Etiology and Prevention	13	$2,055,510	8	$1,239,181	5	$409,254	7	$396,086	33	$4,100,031
Solid Tumor-Virus	11	3,461,697	7	1,426,577	1	113,906	3	497,167	22	5,499,347
Special Animal Ecology Studies	8	837,975	8	1,472,673	3	379,934	2	130,687	21	2,821,269
Program Resources and Logistics	15	2,172,716	11	446,290	5	164,000	4	561,313	35	3,344,319
Biohazards and Containment	2	1,545,034	1	80,000	-	---	-	---	3	1,625,034
Total	49	10,072,932	35	4,664,721	14	1,067,094	16	1,585,253	114[1/]	17,390,000

[1/] Since many contracts include more than one activity, the totals of all catagories do not correspond with the total number of contracts. Actual number of contracts is 102.

4. PROBLEMS AND OPPORTUNITIES

Scientific -- It is entirely probable that within FY 68 - 69 we will be able to conduct sero-epidemiological surveys in the SVLP and STVP to determine whether candidate virus, virus antigen or specific antibody are consistently and predominately associated with leukemia, lymphoma, and 'solid' tumors and/or whether the amount is significantly different from that of normal and disease controls. Successful production and purification of virion-free T antigens now make it possible to test selected human sera for the presence of specific antibodies to adeno T antigens. These antigens and sera have already been collected and it is anticipated that serological studies will begin in early FY 68. Similarly, successful production and purification of several different isolates of the Herpes type virus have made it possible to conduct ongoing studies to determine which of nine different serological tests most effectively detects and quantitates the HTV antigen or antibody in human materials. It is anticipated that comparative tests will be done within FY 68, which will then make it possible to screen large numbers of human sera and tissue for the presence and amount of antibody or antigens. Unlike the adenoviruses within the Solid Tumor-Virus Program Segment, investigators of the Special Virus-Leukemia Program do not yet know how many different major antigens are associated with the Herpes type viruses isolated from over 60 leukemia and lymphoma patients. The definitive serological survey therefore must await this determination.

The major opportunity for significant advancement of the SVLP in 1968 - 1969 is the attainment of definitive information to determine whether this Herpes type virus(es) is etiologically related to Burkitt lymphoma - and perhaps to other human neoplasms as well. The test clinical materials, reagents, facilities and personnel, and serological procedures are now available for this study.

Since the assumption that at least one virus is an indispensable element for the induction of human leukemia and lymphoma is accepted as an hypothesis, current obstacles to prevention of the disease are clearly definable. Requisite sequential steps in the vaccine control of any infectious disease (by bacteria, viruses, etc.) remain the detection, isolation, characterization and replication in large quantities of the causative agent(s). If the viruses already isolated from patients with leukemia are, in fact, causally related to the disease, then the following principal problems remain to be solved prior to the development and test of an experimental vaccine. First, we must scale-up virus production from the relatively small laboratory quantities needed for individual experiments to very large industrial quantities without sacrificing the strength and purity of the agent. This will allow mandatory studies into means for killing the virus prior to its use as a safe but effective vaccine. As with polio for which 3 types of virus are required for complete vaccine protection, large scale production will allow us to determine how many different virus types must be included in a test vaccine. Work currently ongoing will provide at least a part of the quantities of virus needed for these studies within FY 68.

Second, we must gain reasonable proof that the viruses are in fact related to the causation of leukemia in man. It is not possible to show this directly since man will not be used as a test animal. Extensive tests in newborn monkeys, now being done, may provide indirect suggestive evidence. Another adjunctive means for obtaining proof of a cause and effect relationship is to test the bloods of thousands of normal and diseased persons. for the differential presence and amount of the suspect virus or virus product (antibody). The mechanics of this sero-epidemiological survey are being developed. Reasonable direct proof of the association of a virus(es) with human leukemia is necessary also because of the unusual time and expense which will be required to evaluate the effectiveness of test vaccines prepared from this virus(es). Because leukemia and lymphoma are relatively uncommon diseases, and because the first peak incidence of disease does not occur in children until 5-7 years of age, it may be necessary to conduct the first controlled field trial in 1/2 to 1 million people. We anticipate, therefore, that definitive evaluation of test vaccines in man will require approximately 5-7 years and $30,000,000.

Third, in order for man to be protected against virus-induced malignancies, he must develop and maintain an adequate amount of specific antibodies to the causative viruses or their specific antigens. We must determine in animals, therefore, the kind and amount of vaccine, the schedule of inoculation, and other variables to insure the needed level and maintenance of resistance. An even more fundamental problem regarding the possible use of viral vaccines concerns the phenomenon of tolerance. If, for example, "leukemia viruses" of man, as in murine systems, are transmitted vertically, very young children would be expected to have some or complete tolerance to the viral antigens. If this is true, and unless safe procedures are found for breaking tolerance, it simply may not be possible to elicit protective antibodies to the vaccine. A significant amount of work is being done on this problem in murine systems at the present time.

Fourth, we have not yet developed a sensitive animal or tissue culture indicator for determination of infectivity and disease induction capabilities of either the "C" type or Herpes type viruses. This must be done not only as a guide for the detection and measurement of oncogenic activity but also as a means for determining vaccine safety.

Fifth, perhaps one of the most serious obstacles to the prevention of virus-induced human cancers with vaccines is the possibility that some malignancies may be induced not only by so-called tumor viruses, but also by common ubiquitous viruses in combination with environmental, physical or chemical cocarcinogens. The studies of Kotin, et al on the induction of lung tumors in mice following installation of influenza virus and fractions of ozonized gasoline is one example of this phenomenon. If this phenomenon applies to man. it may be very difficult, perhaps impossible, to prevent more than a relatively few cancers with specific "tumor virus vaccines."

Management -- The common denominator to virtually all management problems is time. The Viral Oncology Program now includes 102 contracts, each of which must be managed individually, as well as integrated within the total program. Effective scientific and administrative management of these contracts requires an extraordinary amount of time by project officers and program managers. The time and effort required by this activity is frequently provided at the expense of intramural research activities. The acquisition of new laboratory space will allow recruitment of additional scientists who are expected (and told at the time of recruitment) to share in this management responsibility. This, however, is only a partial answer since it nearly invariably takes one to three years before a new recruit becomes an effective project officer. The most effective means we have found for handling the workload are: (a) Take full advantage (to the point of exploitation) of the high level of administrative competence of our contract specialists, in particular Thomas Lewin and Fred Shaw, and in some cases, of their very real understanding of certain levels of the science, (b) recruit carefully selected professionals as full time program managers, rather than as part time managers and part time laboratory workers (some of these people are effectively carrying five to fifteen contracts), (c) arrange for relatively frequent trips of contract personnel to NIH rather than vice versa, (d) hold one joint chairmen's meeting every month to assess the administrative and operational aspects of the total program; hold one joint chairmen's Core Group meeting per month to assess and handle program problems, principally those concerned with contracts, and hold one scientific meeting per month at which time different groups of contractors are invited as speakers, discussants or listeners, and (e) the establishment of a very effective Program Analysis and Communications Section, which, among other things, serves as the central repository and clearing house for all triannual progress reports, notification of due dates, and regurgitation of scientific and operational information and policy to all program personnel.

Probably the most important of all these factors is the recruitment of knowledgeable, objective, energetic and thick-skinned professionals who are willing to perform as full time program managers. These positions can be made to be challenging and intellectually rewarding. They must also, as was stated in the Ruina Report, be as financially rewarding, as visible, and as respected as that associated with being a laboratory scientist. It is, in fact, the balance between the program manager's operational activities and the scientist's research activities, which must be established and maintained for effective program. While we have made significant progress toward attaining this balance, newer, more efficient means and people must be found to effectively handle the increasing workload.

Finally, and in particular, the selfless dedication and skill of the Segment Chairmen and Vice-Chairmen and their associates, and of W. Ray Bryan as Scientific Coordinator in Virology to the planning and implementation of all facets of the Program, are of such self-identifying stature as to require a simple, dignified acknowledgement.

PUBLICATIONS - Office ASDVO

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Hellman, A., Farrelly, J. G., and Martin, D. H. Some biological properties of DMSO. Nature, 213: 982-985 (1967).

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CONTRACT NARRATIVES
SPECIAL VIRUS-LEUKEMIA PROGRAM
ETIOLOGY AREA, NCI
Fiscal Year 1968

DEVELOPMENTAL RESEARCH PROGRAM SEGMENT

Dr. Robert A. Manaker, VBB, Etiology Area, Chairman
Dr. Albert J. Dalton, Chief, VBB, Etiology Area, Vice Chairman

PRESBYTERIAN-ST. LUKE'S HOSPITAL (PH43-62-179)

Title: Virus-Cancer Research in Marmosets

Contractor's Project Director: Dr. Friedrich Deinhardt

Project Officers (NCI): Dr. Frank J. Rauscher, Jr.
Dr. Roy Kinard

Objectives: To evaluate marmosets as laboratory animals in tumor virus research.

Major Findings: Malignant sarcomas were induced in marmosets by injection of the Schmidt-Ruppin strain of Rous sarcoma virus (SR-RSV) extracted from chicken tumors. The SR-RSV passaged repeatedly by Dr. Howard Temin in cultures of chicken embryo cells was not oncogenic in marmosets. The avian virus could be recovered from some cultivated marmoset tumor cells when disrupted chicken embryo cell material was added. No responses have been observed in marmosets that were inoculated with human leukemic bone marrow, Burkitt's lymphoma materials or the Moloney strain of mouse sarcoma virus.

Significance to Biomedical Research and the Program of the Institute: This project was initiated to determine whether a small, relatively inexpensive lower primate would be suitable for the detection of and/or responsive to viruses associated with oncogenesis in man. A sensitive experimental animal would be invaluable for immunological studies and eventually vaccine safety tests.

Proposed Course: The study of virus-induced neoplasia in the marmoset with known oncogenic viruses will continue. Selected viruses associated with human neoplastic disease or of potential significance in oncogenesis will be tested for activity in the marmoset.

Date Contract Initiated: March 15, 1962

Current Contract Level: October 1, 1967 - September 30, 1968: $166,655

BAYLOR UNIVERSITY (PH43-63-590)

Title: Studies on Viruses as Related to Cancer with Emphasis on Leukemia

Contractor's Project Director: Dr. Joseph Melnick

Project Officers (NCI): Dr. Frank J. Rauscher, Jr.
Dr. Roy Kinard

Objectives: To isolate, propagate, and identify viruses for evidence of their association with human leukemia. Further, to supervise the management of a baboon breeding colony, and to use these animals for tests with viruses suspected to induce cancer in humans.

Major Findings: Application of the indirect immunofluorescence technique showed a high frequency of reaction between sera from leukemic children and their contacts and the surface membranes of cultured Burkitt's lymphoma cells. Study of the mechanism of the reaction showed that the system does not measure combination of immunoglobulin with the cell membranes, but rather the combination of β1C globulin, the third component of complement.

Propagation of bone marrow cells from children yielded fibroblastic outgrowths. Lymphoblastoid cells also grew in some cultures, predominantly in those initiated with tissue from children with infectious mononucleosis. Cultures of similar cells from healthy donors were difficult to initiate and to perpetuate. Herpes-type virus was detected in 3 of 6 lymphoblastoid lines after cultivation on a feeder layer of normal human embryo fibroblasts only. Other viruses were not detected.

Baboons for use in this project are provided from a colony maintained at the Southwest Foundation for Research and Education. Baboon fetuses were inoculated at mid-gestation with fresh cells and plasma drawn from the peripheral circulation or bone marrow of children either with untreated acute leukemia or in relapse. Burkitt's lymphoma cells and infectious mononucleosis materials were similarly injected. Other known human viruses which may have oncogenic properties were inoculated into newborn baboons. Groups of animals were given different immunosuppressive treatments in these tests. The animals are under observation.

Significance to Biomedical Research and the Program of the Institute: This is a well-planned and well-balanced progressive investigation to ascertain the role of viruses in human leukemia. While accomplishing laboratory work in electron microscopy, immunology, tissue culture and primate inoculation, this group has never lost contact with the clinical aspects of human leukemia and have consistently directed their efforts towards the objectives of the SVLP, to prevent human leukemia.

Proposed Course: Studies will be expanded to include studies on the role of DNA viruses in cancer, specifically to include research on cytomegaloviruses and Burkitt herpes type viruses. The critical appraisal of the indirect membrane immunofluorescence test will be continued. Studies with lower primates will be expanded to determine the response of animals held for long periods in a controlled radiation environment.

Date Contract Initiated: June 27, 1963

Current Contract Level: February 14, 1967 - January 31, 1968: $689,700

HEALTH RESEARCH, INC. (PH43-63-593)

Title: Electron Microscopy and Tissue Culture Studies of Viruses in Leukemia Cells, Tissues, and Plasma

Contractor's Project Director: Dr. James T. Grace, Jr.

Project Officers (NCI): Dr. Albert J. Dalton
Dr. Victor Zeve

Objectives: Current objectives are to conduct studies on the herpes-type viruses associated with human leukemias and lymphomas, to use electron microscopy to select materials from leukemia and lymphoma patients which contain virus for further study, and to provide a monitoring service to detect contamination of all cultures by mycoplasma.

Major Findings: Cell-free preparations of herpes type virus from cultured cells derived from Burkitt's lymphoma were shown to be infective for cultured, virus-free lymphoblastoid cells. With a multiplicity of 1000 virus particles per cell, virus attachment, maturation and release was followed by a combination of electron microscopic and immunofluorescent observations. Virus release was observed 65 hours after infection and all cells were dead at 120 hours. At low multiplicities of infection, chronically infected cultures were obtained which continued to release virus as do Burkitt tumor cell cultures. Virus was found to absorb to some lymphoid cells from the peripheral circulation, spleen and lymph node of man. Virus absorption was also noted to occur with some cells from rhesus monkey spleen and lymph node, but not to cells from the thymus. When lymph node cells from rhesus monkeys were incubated with virus *in vitro* and reintroduced into the donor, the animals developed immunofluorescence antibodies to the agent. Cultured cells other than human or rhesus lymphoid cells failed to absorb virus. Although kitten brain tissue responded negatively, kittens developed neurogenic disease following intracerebral injection of virus. Some surviving cats developed a lymphocytosis.

Ferritin-labeling methods showed that IgG from human sera positive by immunofluorescence did coat virus particles. The method was used to show similar antibodies in sera from Burkitt lymphoma patients and a healthy American. Micro double gel diffusion precipitation tests showed precipitating antibody in about 4 percent of sera from 48 healthy donors and in 44 percent of Burkitt's lymphoma patients. Positive sera had titers greater than 500 in immunofluorescence tests. Immunofluorescence tests using goat anti-human IgG and IgM in the indirect method on sera from 13 patients with a diagnosis of infectious mononucleosis showed 7 with high and 2 with low titers on single test while 2 patients exhibited higher and 2 lower serum titers on later bleeding.

Significance to Biomedical Research and the Program of the Institute: The close association of the herpes type virus with hematopoietic tissue in man necessitates a complete evaluation of its role, if any, in leukemia and lymphoma. Advances made by this group contribute considerably to the characterization and the biological activity of this agent in direct pursuit of the objectives of the SVLP.

Proposed Course: The investigators propose an aggressive pursuit of data on the range of biological activity of the herpes type viruses from lymphoma of man and determination of host disease responses to these agents through continued laboratory investigation and limited epidemiological surveys.

Date Contract Initiated: April 18, 1963

Current Contract Level: July 1, 1967 - June 30, 1968: $158,000

UNIVERSITY OF MICHIGAN (PH43-65-639)

Title: Research on Virus Particles in Human Plasma and Tissue Culture Specimens

Contractor's Project Director: Dr. William H. Murphy

Project Officer (NCI): Dr. Mary A. Fink

Objectives: To isolate, characterize, and identify virus-like particles from specimens from leukemia and lymphoma patients, specifically particles of the C-type. Immunofluorescence methods will be used to search for viral antigens in leukemic marrow specimens. Further, to attempt detection and isolation of defective candidate leukemogenic viruses in human cells using selected viruses as helpers in combination with established human indicator cell lines.

Major Findings: Examination of 26 clinical specimens from leukemia patients by electron microscopy showed virus-like particles in 4. Direct isolation of herpes simplex and adeno-12 viruses was accomplished from 2 of 71 bone marrow aspirates from leukemic children. Unidentified agents producing marginal cytopathogenic changes were detected when leukocyte cultures were co-propagated with established human indicator cell lines. One of 11 leukemic children and 5 of 71 non-leukemic children were positive. Similar effects were observed in 4 of 19 leukemic adults and in one instance herpes simplex virus was identified.

Studies on distribution of C type particles in several strains of mice by electron microscopy showed maximal concentrations in germinal centers of the spleen.

For virus genome retrieval studies, human cell lines susceptible to infection by avian and mouse oncogenic viruses and by human myxovirus of potential "helper" value were selected. Propagation of the "helper" viruses in the human cell lines was observed and the significance of these observations is under investigation. Confirmation of the identity of the cell lines and the presence of virus-specific antigens in affected cultures remains to be determined.

Significance to Biomedical Research and to the Program of the Institute:

Confirmation of the results obtained will advance studies on the rescue of virus from human cells in which virus may be present but incapable of maturation to an infective particle. Effective rescue of an RNA genome from neoplastic human cells as is possible in certain animal tumors would be of considerable import in studies on the human disease.

Proposed Course: The investigator proposes to verify his observations; the contract is expected to be reduced from present levels.

Date Contract Initiated: June 21, 1965

Current Contract Level: $219,918

CHICAGO PARK DISTRICT (LINCOLN PARK ZOO) (PH43-65-1017)

Title: Marmoset Breeding Colony

Contractor's Project Director: Dr. Lester E. Fisher

Project Officer (NCI): Dr. Roy Kinard

Objectives: To provide marmosets in a quantity and of a quality sufficient for the research on tumor viruses conducted under contract no. PH43-62-179 at Presbyterian-St. Luke's Hospital, and to provide a separate isolated breeding colony because marmosets are subject to fatal epidemics.

Major Findings: A basic colony of 139 acclimated marmosets and an accessory group of 18 animals, all adults and sub-adults in good health has been achieved.

Significance to Biomedical Research and to the Program of the Institute:

This contract is part of a major NCI program to determine whether viruses are associated with the induction of human cancer. Known animal leukemia viruses do not readily cross species barriers. Therefore, investigations are under way for a practical lower primate that might be susceptible to viruses associated with human leukemia. This project supplies animals for tests for such susceptibility as well as to determine the value of this small, inexpensive primate for cancer virus research.

Proposed Course: The project will be continued to insure availability of experimental animals of quality.

Date Contract Initiated: June 28, 1965

Current Contract Level: February 1, 1968 - January 31, 1969: $29,996

WISTAR INSTITUTE (PH43-65-1028)

Title: Problem Survey of Human Leukemia Virus Test Systems

Contractor's Project Director: Dr. Vittorio Defendi

Project Officers (NCI): Dr. Elizabeth Chu

Objectives: To assess the responsiveness of human hematopoietic tissue in organ culture systems to extracts of human leukemic bone marrow, mycoplasma, and to conjoint effects of the different tissues upon the cellular composition of each in conjoint cultures.

Major Findings: Macrophage cultures infected with the oncogenic viruses, SV40, Schmidt-Ruppin strain of Rous virus (SR-RSV), and the Moloney strain of mouse sarcoma virus (MSV) were studied in regular and conditioned medium. A stimulation of cell proliferation was observed but no evidence of transformation was seen. The grid organ culture system easily yielded human lymphoblastoid cell lines. Two of these lines from normal spleen were exposed to SV40, SR-RSV, and MSV and are under observation. A morphological transformation of lymphoblastoid cells was observed when co-cultivated with RSV-50 transformed hamster cells.

Significance to Biomedical Research and the Program of the Institute: The primary purpose of this contract is to determine whether known animal and candidate human leukemia viruses will replicate and/or induce a detectable response in modified or new culture systems which under general laboratory conditions show little, if any responsiveness to such agents. It is possible that organized tissues, as in organ cultures of different human hematopoietic tissues, will be a more sensitive indicator than more commonly used cell suspensions. In view of the problem in crossing species barriers with a candidate leukemogenic virus, this project is an important and unique complement to other program efforts towards the establishment of replication of virus and/or transformation of cells within *in vitro* systems.

Proposed Course: The results obtained during the current contract year will determine the course of future work by the methods under evaluation.

Date Contract Initiated: June 28, 1965

Current Contract Level: October 1, 1967 - September 30, 1968: $37,540

RUTGERS UNIVERSITY (PH43-65-1039)

Title: Immunogenic Enhancement Studies on Murine Leukemia Viruses

Contractor's Project Director: Dr. Nicholas C. Palczuk

Project Officers (NCI): Dr. Louis R. Sibal
Dr. Frank J. Rauscher, Jr.

Objectives: To complete studies on the enhancement of the immunogenic potency of murine leukemia viruses and herpes group viruses through the use of methylated bovine serum albumin and other enhancing materials and/or procedures.

Major Findings: Freund's adjuvant and S. marcescens endotoxin were effective in enhancing immunogenicity of murine leukemia virus. The persistance of the virus neutralizing antibodies induced in rabbits was longer than when adjuvant 65 was used. Immunogenic enhancement by Bordetella pertussis extract was associated with a heat labile factor. Beryllium sulfate with incomplete Freund's adjuvant was effective in inducing high levels of antibody. In BALB/c strain mice, low levels of serum neutralizing activity were induced with formalized Moloney leukemia virus combined with different adjuvant mixtures. There was no correlation between neutralizing titers and passive hemagglutination titers of rabbit antisera. The absorbed sera reacted with Moloney plasma-derived leukemia virus and with ficin-treated Rauscher plasma-derived leukemia virus in micro gel diffusion precipitin tests.

Adjuvants were evaluated in rabbits in combination with herpes simplex, herpes tamarinus, and infectious bovine rhinotracheitis viruses. Neutralizing titers greather than 500 were obtained and specific complement fixing activity was demonstrated.

Significance to Biomedical Research and the Program of the Institute: The purpose of this project was to evaluate different agents for their effectiveness in enhancing immune response of animals to poorly antigenic viruses. The information would be of value for immunological studies with candidate human leukemia viruses.

Proposed Course: This contract will terminate at the end of the current contract year.

Date Contract Initiated: June 28, 1965

Current Contract Level: $51,305

CHARLES PFIZER AND COMPANY, INC. (PH43-66-98)

Title: Development of Tumor Test Systems

Contractor's Project Director: Dr. J. J. Oleson

Project Officers (NCI): Dr. W. Ray Bryan
Dr. Robert A. Manaker
Dr. John B. Moloney

Objectives: (1) To provide support to NCI Viral Oncology programs; (2) To produce murine leukemia viruses in tissue cultures and in mice; (3) To conduct research in viral oncology as specified by the project officers. In particular, to conduct investigations on C-type animal tumor viruses from tumors initiated with small doses of virus.

Major Findings: Murine leukemia virus production from mouse plasma was continued during the past year. Approximately 105 ml of Rauscher virus and 62 ml of Moloney virus was produced each month. Normal mouse serum, plasma and packed erythrocytes were supplied to support NCI research. Production of Rauscher virus in tissue culture is being re-introduced to supply large demand for NCI projects. As this material becomes available it will replace production of Rauscher virus in mice.

Production of cells and herpes type virus from cultures of the P3J (Jijoye) line of Burkitt's lymphoma cells was increased and presently provides about 50 ml of concentrated virus each week. Quantities of cells of different lines were produced on demand for investigators at NCI and other institutions engaged in research of significance to NCI program goals. Cells produced under another contract (PH43-65-616, Health Research, Inc.) were shipped to the contractor. Lots with high virus content were extracted for virus.

Different methods for virus concentration and purification were evaluated. A technique using fluorocarbon treatment and rate zonal centrifugation has provided concentrates of herpes type virus estimated to contain 10^8 to 10^9 virus particles per ml with a protein content of 50 to 350 gamma per ml.

In collaboration with the Communicable Disease Center, blood, bone marrow and lymph node specimens were collected for tissue culture purposes from the leukemia patients, relatives and close associates in leukemia cluster areas. Six cultures which survive have no virus.

Under the direction of Dr. W. Ray Bryan, a new project was begun in collaboration with other contractors (Germfree Life, Inc., PH43-65-95; Life Sciences, Inc., PH43-68-711; Microbiological Associates, PH43-67-697) to study the recovery of virus from tumors induced by low doses of oncogenic virus. Low dose tumors produce little or no infective virus. Groups of Japanese quail with such tumors are under study.

A total of 2,320 samples were examined by electron microscopy. Of these, 720 were outside requests.

Attempts are under way to repeat observations reported by another laboratory on infectivity of Burkitt's virus for cultured virus free lymphoid cells. Virus uptake by cells was noted but no evidence of the reproduction of virus has been obtained.

Significance to Biomedical Research and the Program of the Institute: This contract was initiated to provide services and applied research to supplement and support intramural research in viral oncology. It was incorporated into the Special Virus Leukemia Program and expanded to include active research and support functions with human materials and associated viruses. It is the major source of services and of cell and virus materials in the large quantities required by participants in the SVLP as well as by other investigators active in research related to Program goals.

Proposed Course: The production of Moloney virus from mouse plasma will continue, but plans have been made to replace Rauscher virus production in animals by a tissue culture production program. Attempts to isolate viruses from human leukemia specimens will continue. Service functions which are the main contribution by this organization will provide electron microscopy and applied research as required. Cell lines of importance will be carried or maintained in frozen storage. Materials significant to SVLP objectives will be made available to investigators as directed.

Date Contract Initiated: November 6, 1961

Current Contract Level: January 1, 1968 - December 31, 1968: $1,684,943

UNIVERSITY OF TEXAS
M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (PH43-65-604)

Title: Studies on the Relationship of Viruses to Human Leukemia and Lymphoma

Contractor's Project Director: Dr. Leon Dmochowski

Project Officers (NCI): Dr. Albert J. Dalton
Dr. Robert A. Manaker

Objectives: To develop and apply electron microscope, tissue culture, and immunological methods to the detection, isolation, propagation and characterization of viruses or viral antigen of potential etiologic significance in leukemia and lymphoma of man.

Major Findings: The examination by electron microscopy of thin sections of lymph node biopsies, blood plasma sediments and tumors as well as tissue culture materials from patients with leukemia, lymphoma or solid tumors showed the presence of particulates resembling virus of the C-type in few specimens. One of 52 cases of leukemia, lymphoma, and Hodgkin's disease was positive for particles in lymph node, 1 of 4 blood plasma pellets contained virus-like material, and 6 of 36 tissue culture preparations had C-type particles which were few in number and not present consistently. A herpes virus was observed in a melanotic tumor.

Tissue cultures of lymph nodes from leukemia patients eventually reduced to a typical fibroblast growth pattern, half of which survived 2 months but few of which lived beyond 6 months. Thirteen cell lines were used for immunological tests by the mixed hemadsorption method to determine whether leukemia, lymphoma, and Hodgkin's disease patients contain antibodies to cells from these diseases. Various serum-cell combinations were used in 545 tests. With 6 of the cell lines, the tests were negative. Four of 6 cell lines from lymphoma patients were positive, especially with sera from patients with lymphoma or Hodgkins disease. However, HeLa cells gave positive reactions with greatest frequency. Heating the serum did not affect a positive reaction. Tests are under way to determine whether mycoplasma contamination is a factor and whether alloantigenic factors are involved.

The Möller membrane immunofluorescence test (MIF) was used in a total of 745 tests with 16 cell lines and 12 buffy coat specimens. Four of 8 cell lines from leukemia patients and 4 of 7 lines from lymphoma patients were consistently negative with all sera tested. Autologous serum-cell combinations with cells and serum from 2 patients showed considerable variation from positive to negative and back.

Significance to Biomedical Research and the Program of the Institute: The Special Virus-Leukemia Program is based in large part on the assumption that viruses similar to those (type C) known to be associated with induction of leukemia in laboratory and domestic animals were the most likely candidates as etiological agents in some human leukemias and lymphomas. At present considerable activity in other laboratories is directed to the elucidation of the role of the herpes type viruses in human cancer. This is one of two contracts whose activities have been restricted by program to the detection, isolation and characterization of the C type particulates associated with human leukemia and lymphoma.

Proposed Course: Electron microscope monitoring will give support to tissue culture investigations and will be used to screen other organs than the lymph node and bone marrow materials which have already been exhaustively probed. Immunological procedures introduced will be evaluated for significance.

Date Contract Initiated: March 19, 1965

Current Contract Level: February 1, 1968 - January 31, 1969: $359,880

BIONETICS RESEARCH LABORATORIES, INC. (PH43-67-661)

Title: Investigation of the Carcinogenic Activity of Selected Virus
Preparations in Newborn Monkeys

Contractor's Project Director: Dr. Arthur J. Pallotta

Project Officers (NCI):
Dr. Frank J. Rauscher
Dr. W. Ray Bryan
Dr. Roy Kinard
Dr. Mary Fink

Objectives: Investigation of carcinogenic activity of selected virus preparations inoculated into newborn monkeys is the purpose of this contract. A major part of this program includes maintenance of an in-house breeding program which has been reduced to produce 300 newborn monkeys a year.

Major Findings: The existing breeding colony of 859 adults of 9 species of primate is being reduced to 500 animals of 5 species. The colony continues to produce well, and the infant mortality rate has been 6.7%. A leukemic monkey from Brooks Air Force Base was examined post-mortem and materials from the animal were inoculated into 11 newborn monkeys. Fifteen cultures of different tissues from the leukemic animal were propagated. A cytopathogenic agent was present. Among the animals inoculated with various materials, a total of 97 newborn macaques were administered fresh whole blood from Clinical Center leukemia patients. Five chimpanzees were inoculated with different human materials. A total of 1,203 inoculated monkeys is in holding. Twelve successful cell cultures were established from the peripheral blood of chimpanzees, both inoculated and uninoculated. Only one of these leukocyte cultures contained a herpes-type virus. Cultures were also initiated from rhesus monkey organs and carried in continuous culture. Selected monkey tissue cultures are being used in an attempt to induce malignant change with suspected oncogenic viruses.

The immunology program undertook production of immune sera in monkeys. Immunosuppressive treatment including Imuran, prednisolone, and x-irradiation was used to condition animals that received inoculations of different leukemia materials.

The collection of parasites from primates is now indexed and cataloged and can be used as a reference for species identification.

Significance to Biomedical Research and to the Program of the Institute:

The difficulties encountered in investigations with suspected oncogenic viruses in man are magnified by the lack of a suitable experimental animal. This project is an attempt to make available a sensitive animal host to the research activities conducted under the Special Virus-Leukemia Program.

Proposed Course: The limitations of animal holding space necessitated a reduction in the breeding colony and in the number of experimental procedures to be conducted in the continuation of this contract.

Date Contract Initiated: February 21, 1962

Current Contract Level: January 21, 1967 - June 20, 1968: $1,719,811

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE (NCI-VCL-67-39)

Title: Irradiation of Neonatal Primates to Evaluate Effects of Irradiation on Susceptibility to Candidate Leukemogenic Viruses.

Contractor's Project Director: Dr. Robert T. Bowser

Project Officer (NCI): Dr. Robert C. Reisinger

Objectives: To determine the effects of mixed gamma-neutron irradiation on the immune competence of newborn primates, and to determine if susceptibility to candidate leukemogenic viruses is enhanced following such irradiation.

Major Findings: Each of four groups totaling 30 infant *Macaca mulatta* (1-7 days of age) was exposed to one of 4 different levels of mixed gamma-neutron irradiation in an attempt to determine an LD 10/60. As anticipated, the infant Rhesus was found to be far more sensitive to the effects of irradiation than is the adult. The higher dosage levels of 340, 320 and 260 rads resulted in a death rate of 71%, 37% and 50%, respectively. The first group (7 animals) receiving 200 rads had a 0% mortality, as did the next two groups (17 animals). However, the next five groups (48 animals) had an $LD_{10/60}$ of 31%, 37%, 50%, 25% and 12%, respectively. Deaths in the groups receiving the higher dosages were caused by invasion of intestinal bacteria following the rapid decline in white blood cells which resulted from irradiation damage. Deaths in the 5 groups receiving 200 rads were attributed to infection with a Pseudomonas organism inadvertently introduced into the nursery, as well as to invasion by intestinal bacteria.

Blood changes following irradiation were similar in all animals. The first change noted was a rapid decline in the number of reticulocytes which reached minimum levels on the 5th and 6th days following exposure. Shortly thereafter, they increased rapidly, reaching maximal values of 14% to 24% fifteen to twenty-three days post irradiation, although the minimum values, maximal responses, and time periods required for regeneration were proportional to radiation dosage. Decline in platelets, leukocytes, and erythrocytes was less rapid, reaching minimal values eleven to fifteen days after irradiation. Leukocytes dropped to 300 to 1,000 per cubic milimeter, platelets to 30,000 to 100,000/c.m.m. and hematocrits to 20-27 percent. Effect of irradiation was variable with regard to the different leukocyte types. Lymphcytes reached extremely low levels within twenty-four hours. Neutrophils responded more slowly, showing minimal values 11 to 15 days post irradiation. Monocytes, eosinophils and basophils also decreased. Following irradiation, signs of recovery were noted in the increasing values of the reticulocyte count by the eighth day, platelets by the eighteenth day, leukocytes between the 15th and 18th day, and the hematocrit by the 18th to 20th day. Lymphocytes were first to show signs of cellular regeneration, and neutrophils and monocytes showed similar signs between 18 and 20 days. Thirty to 40 days post irradiation all parameters of the blood count returned to within reasonably normal levels, but not until 48 to 60 days post irradiation did average lymphocyte values approximate normal control values.

To determine antibody response bovine serum albumin (BSA) 0.625mg was inoculated into animals 24 hours pre- or post-irradiation (200 rads) and into non-irradiated controls, either s.c. in Freund's incomplete adjuvant or i.v. in saline. Contrary to anticipation antibody formation to BSA was appreciably greater in irradiated animals than in the controls. It was also observed that inoculation of tissue culture cells containing herpes-type virus simultaneously with BSA resulted in lower BSA titers than administration of the cells, or human whole blood, 4 or more days following BSA inoculation.

One to nine days following exposure to 200 rads 46 infant Rhesus monkeys were inoculated with either 1) 2-2.5 ml. of packed P-3 cells containing herpes-type virus, 2) human whole blood from leukemic patients, 3) a cell line originated from a human sarcoma, 4) two chimpanzee buffy coat cell lines containing herpes-type virus, 5) Molony sarcoma virus, or 6) Schmidt-Rupin strain of Rous sarcoma virus. None of these animals has so far (10-12 months later) shown signs of tumor development. Thirty-five of the forty-six animals have survived and are being held for further observation.

Significance to Biomedical Research and the Program of the Institute: The response of infant Rhesus monkeys to varying doses of gamma-neutron irradiation, and some of the complicating factors affecting that response have been determined. Irradiation (200 rads) 24 hours before or after inoculation of an antigen (BSA) enhances rather than depresses the immune response to that antigen. If immunosuppression relative to a specific inocula is to be accomplished in newborn Macaca mulatta it appears that determining the optimum time of inoculation relative to irradiation might be more promising than increasing the irradiation dose. If the irradiation dose is appreciably increased somewhat older animals must be utilized. Supportive antibiotic therapy was utilized during thes experiments as clinically indicated, and is deemed necessary in any similar future experiments with newborn monkeys.

Proposed Course: Attention will be directed toward irradiation of older animals, and those previously inoculated.

Date Contract Initiated: January 2, 1967

Current Contract Level: $25,000 (FY 1968)

TESTING AND MONITORING PROGRAM SEGMENT

Dr. Mary A. Fink, VLLB, Etiology Area, Chairman
Dr. James T. Duff, VCB, Etiology Area, Vice-Chairman

CHILDREN'S HOSPITAL OF PHILADELPHIA (PH43-66-477)

Title: Interference and Immunofluorescence Studies with Cell Lines Derived from Leukemias and Lymphomas.

Contractor's Project Director: Dr. Gertrude Henle

Project Officer (NCI): Dr. Richard Malmgren

Objectives: Conduct Studies on Burkitt Lymphoma Cell Lines.

Major Findings: (1) By fluorescent antibody and electron microscopy studies, evidence was accumulated that the immunofluorescence was detecting the Burkitt herpes virus.

(2) A close association between the development of antibody reactive with the Burkitt herpes virus and infectious mononucleosis was demonstrated.

Significance to Biomedical Research and the Program of the Institute: This contract directly serves a major program interest in the demonstration of viruses associated with human leukemia and lymphoma, either by direct means, or, as in these studies, by serological techniques.

Proposed Course: Further seroepidemiological studies will be carried on to acquire more information on the relationship between Burkitt lymphoma and infectious mononucleosis. Efforts to improve culture conditions for replication of the herpes-like virus will continue along with efforts to transmit this agent to other host systems.

Date Contract Initiated: January 1, 1966

Current Annual Level: **$46,240**

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-67-697)

Title: Assay of Animal Tumor Viruses

Contractor's Project Director: Dr. R. M. Madison

Project Officer (NCI): Part I - Dr. W. Ray Bryan
Part II - Dr. Robert Huebner

Objectives: This contract now consists of two parts:
Part I (Project Officer, Dr. Bryan) - This part of the contract represents a continuation of all of the former service type activities previously conducted, including in vivo bioassays of leukemogenic and other RNA tumor viruses, histopathology, inventory, and storage of human and animal specimen materials involved in various SVLP activities, and other services provided under the initial contract.

Part II (Project Officer, Dr. Huebner) - This part of the contract represents new activities involving in vitro systems introduced in May 1967 as a supplement to the initial contract.

Major Findings: Part I - This year has seen an increase in the capacity of the contractor to perform in vivo assays for murine leukemia viruses, and the backlog of specimens has been substantially reduced. The contractor has continued to carry out all of the service activities in a competent manner.

Part II - (1) In vitro assays for the murine leukemia viruses are just getting underway.

(2) BALB/c mice are being held for study in old age. Tumors of these animals are identified and tested for antigens of a murine leukemia sarcoma group by the complement-fixation test. These same materials are being tested for infectious virus and transplantability.

Significance to Biomedical Research and the Program of the Institute:
Part I - This contract represents an extension of the laboratory facilities of NIH scientists in the Special Virus-Leukemia Program who need the services of a bioassay system for murine leukemia viruses, thus saving an enormous amount of time, space, and job positions. It is also used by contractors within the Special Virus-Leukemia Program.

Part II - This part of the contract is significant to the biomedical research and the program of the Institute in that it is a direct extension of the laboratories of the Viral Carcinogenesis Branch in which various problems including the association between solid tumors and leukemias are under investigation.

Proposed Course: It is proposed that both parts of this contract will continue indefinitely to back-up the activities of the SVLP and to enlarge the areas of research of the Viral Carcinogenesis Branch. To this point, efforts to demonstrate competent or defective viruses in non-lymphomatous tumors of BALB/c mice will continue. Also, Statolon, an interferon-inducing drug, will be studied for its effect on the development of tumors in the geriatric mouse. Infection rates, excretion rates, and possible horizontal spread will be determined for the leukemia virus known to be present in significant proportions of mice held in the geriatric study.

Date Contract Initiated: November 15, 1961

Current Annual Level: $520,530

BIONETICS RESEARCH LABORATORIES (PH43-67-661, Phase B)

Title: Immunologic Studies of Human Leukemia Material

Contractor's Project Director: Dr. Morris Goldman

Project Officer (NCI): Dr. Mary A. Fink

Objectives: Evaluation of Immunofluorescent Techniques

Major Findings: (1) The 133 coded serum samples submitted to the contractor as part of the Comparative Serology Study Group have been tested against the four coded cell lines by the Henle immunofluorescence technique. The same serum samples and the same four coded cell lines are in the process of being tested using the Klein immunofluorescent technique.

(2) Antiserums against selected leukemic materials have been produced in the Rhesus monkey by protocols submitted by the Immunology Section of the Viral Leukemia and Lymphoma Branch.

Significance to Biomedical Research and the Program of the Institute: This contract serves as a testing laboratory for the Immunology Subgroup of the Special Virus-Leukemia Program. It is a service contract for the purpose of carrying out the tests judged necessary by the Special Virus-Leukemia Program. Antiserums prepared under contract at this agency are distributed throughout the SVLP and are the principal source of antiserum for studies performed by the Immunology Section of the Viral Leukemia and Lymphoma Branch.

Proposed Course: It is proposed that this contract be continued until the Comparative Serology tests have been completed. At the end of the Comparative Serology Study, a judgment will be made whether to continue this phase of the contract, to divert the contract into other areas of immunological research, or to phase it out.

Date Contract Initiated: March 1, 1967

Current Annual Level: $190,000

BALTIMORE BIOLOGICAL LABORATORIES (PH43-62-839, Phase B)

Title: Development of fluorescent antibody tests for mycoplasma

Contractor's Project Director: Dr. Theodore Carski

Project Officer (NCI): Dr. Mary A. Fink

Objectives: To prepare fluorescent antibody diagnostic reagents for human strains of mycoplasma for distribution within the SVLP.

Major Findings: During this terminal year the contractor has packaged the immunofluorescent diagnostic serums prepared against several strains of mycoplasma, especially those known to be associated with man. These preparations have been turned over to the National Cancer Institute and are now available for distribution to members of the Special Virus-Leukemia Progra

Significance to Biomedical Research and the Program of the Institute: These serums are useful in the monitoring of cell cultures for the presence of contaminating mycoplasma, the presence of which has been mistaken for viruses. They will be used throughout the participating laboratories of the SVLP.

Proposed Course: This contract was terminated in January of 1968.

Date Contract Initiated: June 26, 1965

Current Annual Level: $19,240 - Now terminated.

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY (PH43-65-1002)

Title: Study of role of mycoplasma isolated from human leukemia specimens.

Contractor's Project Director: Dr. Leonard Hayflick

Project Officer (NCI): Dr. Mary A. Fink

Objectives: (1) To determine the role of mycoplasma in the pathogenesis or complication of human leukemic disease;

(2) To serve as a central typing laboratory for identification of mycoplasma species.

Major Findings: This contract was set up primarily to serve as a central laboratory for the identification of mycoplasma species. For the past year there has been a real increase in the use of the contract for this purpose, with many contractors of the Special Virus-Leukemia Program availing themselves of this service.

Significance to Biomedical Research and the Program of the Institute: This serves as a mycoplasma identification center to which any worker within the Special Virus-Leukemia Program can send specimens for identification. As mycoplasma are known to contaminate tissue cultures, it is imperative that the cause of cytopathogenic effects in tissue culture, which might be attributed to virus, be accurately determined.

Proposed Course: This project will continue at Stanford University under Dr. Hayflick's supervision when he transfers to that institution. Included will be the characterization of mycoplasma strains isolated from primates.

Date Contract Initiated: June 26, 1967

Current Annual Level: $42,000

FLOW LABORATORIES (PH43-67-1135)

Title: Virus Laboratory for Cancer Research

Contractor's Project Director: Dr. Raymond V. Gilden

Project Officer (NCI): Dr. Berge Hampar

Objectives: A laboratory is maintained for the identification of potential cancer viruses, tumor virus antigens and their corresponding antibodies in the human and in other animal species. The specific areas on which this contract is concentrating are as follows: 1) adenoviruses, especially of infra-human origin; and 2) herpes viruses.

Major Findings: Goats have been immunized with all strains of the simian adenoviruses. Serums from these animals have been tested by the neutralization technique. Tumor antigens of the oncogenic simian adenoviruses have been tested by the complement-fixation technique and the fluorescent antibody technique. The cytomegalo viruses are being studied especially by plaque assay and plaque-reduction neutralization tests.

Significance to Biomedical Research and the Program of the Institute: This contract serves as a laboratory for the identification of adenoviruses and herpes viruses, each of which contains members known to be important oncogenic agents.

Proposed Course: Continuation of the development of methodology for the study of oncogenic adeno and herpes viruses.

Date Contract Initiated: June 22, 1965

Current Annual Level: $314,300

UNIVERSITY OF KENTUCKY (PH43-67-1140)

Title: Preparation of Antiserum and Antigens for Equine Herpes Viruses

Contractor's Project Director: Dr. John T. Bryans

Project Officer (NCI): Dr. James T. Duff

Objectives: To prepare diagnostic reagents (200 ml volumes) of antiserums and antigens for the following four equine herpes viruses: Kentucky-D, Plummer's LK, Japan TH20, and Kentucky coital exanthema.

Major Findings: Seed material and rabbit antiserum have been prepared for equine herpes virus (A-183), the LK virus of Plummer, and the equine coital exanthema virus. Titrations for virus neutralizing antibody have been completed. Plaque-reduction and complement-fixation assays of the antiserum are in progress.

Significance to Biomedical Research and the Program of the Institute: This contractor is furnishing diagnostic antiserum and stock antigens for the identification of herpes viruses known to be associated with horses. These reagents will become part of a battery used in the identification of herpes viruses found to be associated with tumors.

Proposed Course: After completion of the preparation and testing of the reagents described, the purpose of the contract will have been fulfilled and termination will be in order.

Date Contract Initiated: June 7, 1967

Current Annual Level: $22,824

EPIDEMIOLOGY PROGRAM SEGMENT

Robert W. Miller, M.D., Epidemiology Branch, Chairman

National Center for Health Statistics (FS-(66)-35)

Title: Death Certificates on Childhood Cancer, 1960-64

Contractor's Project Director: Dr. Robert D. Grove

Project Officer(NCI): Robert W. Miller, M.D.

Objectives: To obtain death certificates for all U.S. children under 15 years of age who have died of cancer, 1960-1964. This registry will be used to determine 1) aggregation of cancer in twins or other sibs, 2) geographic clustering by dates of birth or death, 3) the association of cancer with congenital defects, and 4) the frequencies of specific cancers not separately listed in published mortality statistics; the registry will also be used 5) to match against names of children believed to be at unusual risk of cancer; e.g., those given certain vaccines in the past.

Major Findings:

To date the mortality data have revealed that 1) among about 7,000 Maryland children immunized against measles in 1961 with a vaccine containing viruses capable of inducing cancer in lower species, none of the vaccinated children had died of cancer through 1964; 2) a Boston study, disputed in Great Britain, was correct in concluding that when an identical twin develops leukemia under 6 years of age, there is a high probability (1 in 5) that the co-twin will develop the disease soon thereafter; 3) brain tumors and infantile histiocytosis each aggregate among siblings to a greater extent than was previously known; 4) neuroblastoma, a cancer of nerve tissue in children, originates largely in utero and varies over time in certain subdivisions of the U.S. in a fashion which suggests an environmental influence on its occurrence; and 5) certain cancers occur excessively in children with specific congenital defects, clarifying or suggesting associations not previously known.

Significance to Biomedical Research and the Program of the Institute:
At relatively low cost the registry of childhood cancer death certificates will provide many new opportunities for research into the origins of each form of childhood cancer.

Proposed Course: Copies of corresponding birth certificates will be sought from each State. The data from the birth and death certificates will be abstracted and card-punched, lists and tables will be made to obtain the objectives described above.

Date Contract Initiated: FY-1967

Current Contract Level: $8,500

NATIONAL ACADEMY OF SCIENCES-NATIONAL RESEARCH COUNCIL (PH43-64-44)

Title: Investigations of Viral and Other Factors in Cancer Etiology
in Army Veterans

Contractor's Project Director: Mr. Seymour Jablon

Project Officer (NCI): Robert W. Miller, M.D.

Objectives: 1) To study the cancer experience of 23,149 soldiers following World War II in relation to diagnoses made during military service and suspected to increase or decrease cancer occurrence; e.g., infectious mononucleosis, infectious hepatitis and herpetic infections. 2) To study about 3,200 veterans who developed cancer, 1950-1954, to determine if frequencies of certain events during military service, 1942-1944, exceed those for a matched control group; e.g., blood transfusion and yellow-fever vaccination.

Major Findings: None yet

Significance to Biomedical Research and the Program of the Institute:
Will seek through epidemiologic studies a human counterpart for experimental evidence at NCI and elsewhere that certain environmental agents, viruses in particular, are oncogenic.

Proposed Course: Study should be concluded in fiscal year 1969

Date Contract Initiated: June 1, 1965

Current Contract Level: $32,500

RESOURCES AND LOGISTICS SEGMENT

Dr. Robert Holdenried, PRLS, Etiology Area, Chairman
Dr. Harry G. Steinman, PRLS, Etiology Area, Vice Chairman

BALTIMORE BIOLOGICAL LABORATORIES, INC. (PH43-63-1161)

Title: Fluorescent Antibody Studies of Mouse Viruses

Contractor's Project Director: Dr. Roger Wilsnack

Project Officers: Drs. Wallace P. Rowe (NIAID) and Robert Holdenried (NCI)

Objectives: To develop and utilize special fluorescent murine virus reagents.

Methods Employed: General and special methods of tissue culture, virology and immunology.

Major Findings: Rats consistently developed complement fixation titers of 1:160 or greater to Moloney sarcoma virus. These sera also showed neutralizing antibodies. About 75% of AKR leukemia inoculated rats developed CF titers of 1:40 or greater to the homologous antigen.

Five to 25 ml of exudate could be aspirated from each MSV tumor, which on conjugation displayed immunofluorescent staining activity (direct and indirect), complement fixing ability with MSV, and heterologous murine leukemia antigens. MSV antigen in mouse cultures was demonstrated by direct and indirect staining with fluorescent conjugates of rat exudates and MSV tumor sera, respectively.

Significance to Biomedical Research and the Program of the Institute: The antisera produced is used in other SVLP projects for the study of indigenous leukemia in laboratory mice. The contractor successfully produced antisera in weanling rats to Moloney sarcoma virus and to AKR leukemia virus by use of appropriately infected mouse embryo cell cultures inoculated into suckling rats and implanting the resultant tumor cells into the weanling rats.

Proposed Course: Processing and characterization of sera from rats will be continued. The application of the immunofluorescent technique to quantitation of leukemia virus and virus antibody by fluorescent plaque count and fluorescent plaque neutralization, respectively.

Date Contract Initiated: June 2, 1963

Current Annual Level: October 1, 1967 - September 30, 1968: $79,664

BALTIMORE CITY HOSPITALS (PH43-66-908)

Title: Human Fetal and Perinatal Tissue Procurement

Contractor's Project Director: Dr. Abou D. Pollack

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: To supply approximately one and one-half pairs of human perinatal kidneys per week, to be trypsinized into single cell suspensions. The processing of the kidneys is performed under contract with Melpar, Inc.

Methods Employed: In appropriate cases of perinatal death, kidneys are removed from the cadavers in a sterile manner. They are minced, placed in tissue culture media and shipped to Melpar, Inc. for processing.

Major Findings: In 1967 the contractor had produced approximately 50 pairs of perinatal kidneys.

Significance to Biomedical Research and the Program of the Institute: The contractor is one of 12 procurement centers which constitute the Tissue Procurement Program of the NCI, a program which procures, stores and distributes human cancer tissue as well as normal tissue to investigators in cancer research who are supported by NCI funds.

Proposed Course: This contract will be terminated at the end of the contract period in May 1968.

Date Contract Initiated: June 27, 1966

Current Annual Level: June 27, 1967 - May 31, 1968: $16,357

BEN VENUE LABORATORIES (PH43-68-633)

Title: Packaging of Viral Reagents

Contractor's Project Director: Mr. Stanley L. Morgan

Project Officer (NCI): Dr. Harry G. Steinman

Objectives: To lyophilize (antisera) and ampoule (antigen) simian adeno= and herpes viruses which are necessary for the Special Virus-Leukemia Program.

Major Findings: This contract was initiated late in the year and no material has yet been processed.

Significance to Biomedical Research and the Program of the Institute: To provide investigators engaged in research for the Special Virus-Leukemia Program a number of "reference" and "working" simian adeno- and herpes viruses conveniently packaged.

Proposed Course: To ampoule 6 simian viruses and lyophilize 20 antisera.

Date Contract Initiated: February 21, 1968

Current Annual Level: February 21, 1968 - February 20, 1969: $27,719

CORNELL UNIVERSITY (PH43-67-664)

Title: Production of Seed Materials for Certain Feline Viruses and Canine Herpesvirus

Contractor's Project Director: Dr. James H. Gillespie

Project Officer (NCI): Dr. James T. Duff

Objectives: Preparation of bulk lots of feline viruses (herpesvirus, rhinoviruses, and panleucopenia virus) and canine herpesvirus. Aliquots of these viruses will be sent to Pitman-Moore Division, Dow Chemical Company (Contract PH43-67-657) for preparation of immune sera in goats.

Methods Employed: General tissue culture, virological and serological techniques.

Major Findings: Eight feline rhinoviruses, feline herpesvirus and panleucopenia virus have been prepared in bulk lots and are available for packaging at NCI. Aliquots of each virus have been submitted to Pitman-Moore Division, Dow Chemical Company, for the preparation of antisera. The viruses have been plaque purified or purified by the terminal dilution procedure. Preparation of virus seed material and antisera for canine herpesvirus is in progress.

Significance to Biomedical Research and the Program of the Institute: Reference reagents (virus seed material and antisera) will be available for the majority of the indigenous feline viruses isolated in this country. These reagents will be useful to investigators working with this animal species in monitoring specific pathogen-free cat colonies, field specimens being inoculated into cats, and in the identification of new viral isolates from these animals.

Proposed Course: Completion of work and termination of contract on June 30, 1968. Further testing and characterization of these viruses and antisera prepared against them will be conducted under Contract PH43-65-620 at Cornell University.

Date Contract Initiated: February 20, 1967

Current Annual Level: February 2, 1967 - June 30, 1968: $6,800

DOW CHEMICAL COMPANY (PH43-67-657)

Title: Feline Virus Antigens and Antisera Preparation

Contractor's Project Director: Dr. James L. Bittle

Project Officer (NCI): Dr. James T. Duff

Objectives: Preparation and testing of bulk lots of antisera against feline viruses (rhinoviruses, herpesvirus and panleucopenia virus) prepared by Cornell University (Contract PH43-67-664). Prepare and test virus seed material and antisera for approximately five feline viruses isolated at this laboratory.

Methods Employed: General tissue culture, virological and serological techniques.

Major Findings: Goats are being immunized with various feline viruses prepared by Cornell University at this laboratory. All viruses have been plaque purified or purified by the terminal dilution method.

Significance to Biomedical Research and the Program of the Institute: Reference reagents (virus seed material and antisera) will be available for the majority of the indigenous feline viruses isolated in this country. These reagents will be useful to investigators working with this animal species in monitoring specific pathogen-free cat colonies, field specimens being inoculated into cats, and in the identification of new viral isolates from these animals.

Proposed Course: The antisera prepared against the various feline viruses will be tested for homologous and heterologous titers. The bulk lots of viruses and antisera will be sent to NCI for packaging and distribution.

Date Contract Initiated: February 20, 1967

Current Annual Level: February 20, 1968 - February 19, 1969: $23,000

FIELD MUSEUM OF NATURAL HISTORY (PH43-65-1040)

Title: Monograph Preparation on Marmoset Taxonomy

Contractor's Project Director: Mr. Philip Hershkovitz

Project Officer (NCI): Dr. Robert Holdenried

Objectives: To complete manuscript on the taxonomy of marmosets.

Methods Employed: Gather and organize data from literature, museum records and field work. Prepare illustrative material. Analyze and synthesize information into a logical taxonomic scheme.

Major Findings: Marmoset classification falls into a logical taxonomic scheme.

Significance to Biomedical Research and the Program of the Institute: The proposed manuscript will assist in obtaining proper identification for marmosets used in cancer research. Only with properly identified animals can the significance of the research be determined and repetition of the experiments be possible.

Proposed Course: The manuscript will be completed and ready for publication in 1968. The present contract will terminate on September 30, 1968. The National Library of Medicine has awarded Mr. Hershkovitz a research grant for publication support of the monograph.

Date Contract Initiated: June 1965

Current Annual Level: The contract was extended to September 30, 1968, without additional funding. Total amount for the contract period of 40 months: $30,000.

FLOW LABORATORIES, INC. (PH43-65-1012)

Title: Low Temperature Specimen Repository Facility

Contractor's Project Director: Mr. J. Walker

Project Officer (NCI): Miss Mary K. Asdell

Objectives: To provide a central repository and shipping facility for reagents and materials necessary for the Special Virus-Leukemia Program.

Major Findings: Approximately 256 orders for viral reagents and allied products have been shipped to domestic and foreign scientific researchers during the past year. Adequate emergency power in case of a power failure has been provided during the past year. All "feeding" contracts for the repository are terminated except Hazleton (PH43-65-635, Rauscher leukemia virus production) and Pfizer (PH43-66-98, Moloney leukemia virus production). Influx of products from the terminated contracts has increased the storage volume by 300%.

Significance to Biomedical Research and the Program of the Institute: A research program, to run efficiently, must have easy access to adequately characterized resources. The storage and shipping facilities of this contract enables the scientist to have a central facility available with a large inventory of materials, while at the same time not overburdening his limited freezer space.

Proposed Course: The level of "outgoing" activity is projected at 300-400 shipments per year. Additional floor space and electrical power will be requisitioned for the proposed expansion.

Date Contract Initiated: June 22, 1965

Current Annual Level: July 1, 1967 - June 30, 1968: $36,223

GEORGETOWN UNIVERSITY (PH43-67-1134)

Title: Operation of a Tissue Procurement Facility

Contractor's Project Director: Dr. Abner Golden

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: To aseptically procure, store, and distribute thoroughly characterized human normal and tumor tissue to NCI affiliated cancer research programs.

Methods Employed: After proper permission is obtained, sterile autopsies and surgicals provide tissues which are processed, characterized, and stored for distribution. Processing includes preparation in nutrient media at 4°C, at -70°C, or in nutrient media and dimethylsulfoxide at -190°C. Characterization of these tissues includes thorough histologic diagnosis of each organ as well as a complete autopsy protocol on the case, microbiologic screening for bacteria and fungi, and viability testing by tissue culture.

Major Findings: In 1967 tissue was obtained from 83 cases. There were 562 specimens shipped to recipients. This contract provides most of the non-neoplastic tissue for the Tissue Procurement Program.

Significance to Biomedical Research and the Program of the Institute: The contractor is one of twelve procurement centers which constitute the Tissue Procurement Program of the NCI, a program which procures, stores and distributes human cancer tissue as well as normal tissue to investigators in cancer research who are supported by NCI funds.

Proposed Course: This contract will be terminated at the end of the contract period in October 1968.

Date Contract Initiated: June 28, 1962

Current Annual Level: May 5, 1967 - October 31, 1968: $115,500

GERMFREE PRODUCTS, INC. (PH43-64-533)

Title: Production of Murine Leukemia Virus Seed Stocks

Contractor's Project Director: Dr. Wendall M. Farrow

Project Officer (NCI): Dr. Michael A. Chirigos

Objectives: To produce 10x plasma concentrates and spleen extracts from mice inoculated with one of 8 murine leukemia viruses.

To produce plasma and spleen concentrates from uninoculated normal control mice.

To breed and maintain a colony of SPF/Balb/c mice to meet work requirements.

Methods Employed: Virus harvests obtained from inoculated mice were partially purified by differential centrifugation. Infectivity of each virus harvest lot was determined by mouse inoculation.

Major Findings: Sizeable amounts of Moloney, Breyere-Moloney, Gross, Buffett, Manaker, Rich, Friend and Rauscher virus concentrates were produced.

Significance to Biomedical Research and the Program of the Institute: Adequate reserve quantities of Breyere-Moloney, Friend and Rauscher viruses have been produced and are available for use in cancer research.

Proposed Course: Terminated.

Date Contract Initiated: March 12, 1964

Current Annual Level: June 1, 1967 - January 31, 1968 (8 months): $217,900

HAZLETON LABORATORIES, INC. (PH43-65-635)

Title: Production of Murine Leukemia Virus

Contractor's Project Director: Dr. Richard A. Pledger

Project Officers (NCI): Drs. Sheldon K. Gottlieb and Harry G. Steinman

Objectives: To produce Rauscher leukemia virus in the appropriate strain of mouse and prepare it as 10x plasma concentrate and 10% spleen extract.

Methods Employed: Virological procedures for partial purification of virus from plasma and spleen of inoculated mice.

Major Findings: Adequate amounts of Rauscher spleen and plasma virus were produced.

Hepatitis virus-contamination has been successfully avoided by holding mice for a given period of time before using them.

Significance to Biomedical Research and the Program of the Institute: A good quantitative and qualitative supply of Rauscher plasma virus and Rauscher spleen virus is being produced for use in cancer research.

Proposed Course: To continue the high production level and insure a clean product.

Date Contract Initiated: June 4, 1965

Current Annual Level: October 1, 1967 - September 30, 1968: $208,880

HEALTH RESEARCH, INC. (PH43-63-43)

Title: Tissue Procurement Facility

Contractor's Project Director: Dr. James T. Grace

Project Officer (NCI): Dr. Robert H. Depue

Objectives: To aseptically procure, store and distribute thoroughly characterized human tumor tissue to NCI affiliated cancer research programs.

Methods Employed: After proper permission is obtained, sterile autopsies and surgicals provide tissues which are processed, characterized, and stored for distirubtion. Processing includes preparation in nutrient media at 4°C, at -70°C, or in nutrient media and dimethysulfoxide at -190°C. Characterization of these tissues includes thorough histologic diagnosis of each organ as well as a complete autopsy protocol on the case, microbiologic screening for bacteria and fungi, and viability testing by tissue culture.

Major Findings: This center provides the tumors for distribution in the Tissue Procurement Program. In 1967 tumors were obtained from 191 cases. There were 607 specimens shipped to recipients.

Significance to Biomedical Research and the Program of the Institute: The contractor is one of twelve procurement centers which constitute the Tissue Procurement Program of the NCI, a program which procures, stores and distributes human cancer tissue as well as normal tissue to investigators in cancer research who are supported by NCI funds.

Proposed Course: This contract will be terminated at the end of the contract period in June 1968.

Date Contract Initiated: August 6, 1962

Current Annual Level: July 1, 1967 - June 30, 1968: $142,032

HEALTH RESEARCH, INC. (PH43-65-616)

Title: Culture of Human Hematopoietic Cells and Large-scale Production of Associated Virus

Contractor's Project Director: Dr. George E. Moore

Project Officers (NCI): Drs. Robert Manaker, Harry G. Steinman, and A.J. Dalton

Objectives: Continue as in the past a program to produce virus (herpes-type) of acceptable quality, free of extraneous bacterial and viral agents, from each of 5 cell lines. To produce virus preparations with a minimum of 10^9 virus particles per ml of suspension from cultures of selected cell lines.

To maintain, by refrigeration, sufficient quantities of each of the 5 cell lines selected for virus production to allow immediate replacement of cultures lost through laboratory procedures or accidents.

Methods Employed: Cell lines have been derived from leukocytes from patients with various leukemias, Hodgkin's disease, other lymphomas, various carcinomas and normal persons. Cell lines for large-scale expansion were transferred to large 200 liter stainless steel culture units where they are grown as suspension cultures. Smaller units are also used for growth of starter cultures. Virus harvest methods consist of centrifugation of the cell cultures.

Major Findings: With the completion of the cell production plan in August 1967, efforts have been concentrated on the production of a large number of different cell lines and the concentration of a herpes-type virus which is being produced. About 240 ml of packed cells are produced a month. The virus content of each lot, as determined by other laboratories, has been varied.

Significance to Biomedical Research and the Program of the Institute: A large portion of current studies conducted within the Special Virus-Leukemia Program is concerned with the determination of: (1) whether the herpes-type virus found to be associated with many leukemia and lymphoma patients in this country and abroad is etiologically related to these diseases and (2) the desirability and feasibility of producing test vaccines of adequate potency, purity and safety. In order to conduct studies toward these program objectives, it is essential that adequate quantities of various isolates of this virus be made available to program investigators.

Proposed Course: There is increasing evidence that the procedures employed by this contract will not result in the production of the quality and quantity of herpesvirus required. The system should be adequately tested by November 1968, at which time termination has been scheduled.

Date Contract Initiated: April 19, 1965

Current Annual Level: June 1, 1967 - May 31, 1968: $373,850

HEKTOEN INSTITUTE FOR MEDICAL RESEARCH (PH43-66-903)

Title: Human Fetal and Perinatal Tissue Procurement Center

Contractor's Project Director: Dr. Maurice A. Mufson

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: To procure approximately three pairs of human perinatal kidneys per week and to ship them to Melpar, Inc. for processing into single cell suspensions.

Methods Employed: In appropriate cases of perinatal death, kidneys are removed from the cadavers in a sterile manner. They are minced, placed in tissue culture media and shipped to Melpar, Inc. for processing.

Major Findings: In 1967, specimens were obtained from 130 perinatal cases. One hundred and forty-three specimens were shipped. In addition, the contractor has performed sterility testing on the kidneys as well as to study the cells for the occurrence of cytopathic and noncytopathic viruses.

Significance to Biomedical Research and the Program of the Institute: The contractor is one of 12 procurement centers which constitute the Tissue Procuement Program of the NCI, a program which procures, stores, and distributes human cancer tissue as well as normal tissue to investigators in cancer research who are supported by NCI funds.

Proposed Course: This contract will be terminated at the end of the contract period in August 1968.

Date Contract Initiated: June 16, 1966

Current Annual Level: June 16, 1967 - August 31, 1968: $34,500

HOSPITAL FOR SICK CHILDREN (PH43-65-97)

Title: Human Leukemic and Normal Tissue Collection and Preservation

Contractor's Project Director: Dr. John M. Darte

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: To obtain serum and plasma specimens from pediatric leukemic patients and nonleukemic controls, storing and making them available for a wide variety of research purposes.

Major Findings: During 1967 specimens were collected from 225 cases and 85 vials of plasma or serum were distributed.

Significance to Biomedical Research and the Program of the Institute: This is a resource contract for the supply of serum specimens from leukemic and nonleukemic children for study by scientists participating in the Special Virus-Leukemia Program in a search for viruses associated with human leukemia. This material will be widely used in immunologic studies.

Proposed Course: Continue to collect serum specimens.

Date Contract Initiated: February 3, 1965

Current Annual Level: May 1, 1967 - May 31, 1968: $7,895

HYLAND LABORATORIES (PH43-66-539)

Title: Serum Testing Laboratory

Contractor's Project Director: Dr. L.E. Gaeta

Project Officer (NCI): Dr. Charles W. Boone

Objectives: To provide three different types of testing activity in relation to approximately 20 samples per month of serum obtained from the Serum Procurement Facility (Contract PH43-66-539, Hyland Laboratories) and from the Viral Carcinogenesis Branch.

Methods Employed: The following is a summary of the types of testing activities being performed:

Category I. Quality Control
- Sterility (viruses, mycoplasma, bacteria, fungi)
- Adulteration control (proof against addition of salt solutions, antibiotics, and foreign sera)
- Hemolysis control

Category II. Characterization Studies
- Growth supporting capacity (heteroploid and diploid cells)
- Immunoelectrophoresis
- Lactic dehydrogenase
- Lipids
- Spectrographic analysis
- Gamma globulin content
- Virus content (BVD, IBR, P13, interference assay)

Category III. Special Projects
- Testing serum from outside sources (7 different national suppliers)

Major Findings: Fetal calf serum samples are being procured twice monthly from 7 major suppliers in the United States, identifying marks removed, and the samples sent to the contractor for testing. It is anticipated that the accumulation of test data on these samples, in addition to the test data on the contractor's own specially procured unfiltered serum, will provide a basis for (1) establishing the normal values and standard deviations for a number of fetal calf serum constituents of concern to scientists using tissue culture, (2) assessing the quality of fetal calf serum produced by national suppliers, and (3) correlating variation in growth supporting capacity with levels of various serum constituents. The contractor is also accumulating data on incidence of various virus inhibitors.

Significance to Biomedical Research and the Program of the Institute: The establishment of a set of test criteria for the quality control of fetal calf serum, in particular the testing for specific bovine viruses, will fill a much-felt need voiced by scientists in the cancer-virus field.

Proposed Course: Two years

Date Contract Initiated: June 24, 1966

Current Annual Level: June 24, 1967 - June 24, 1968: $97,000

HYLAND LABORATORIES (PH43-67-1133)

Title: Facility for the Procurement and Distribution of Sterile, Unfiltered Fetal Calf Serum

Contractor's Project Director: Mr. Larry E. Gaeta

Project Officer (NCI): Dr. Harry G. Steinman

Objectives: To provide fetal calf serum to cell culturists engaged in cancer virology and cell biology research which is of high quality not only with respect to its capacity to support cell growth but also with respect to its freedom from inhibitory adulterants and contaminants, and to ascertain the biochemical parameters which characterize such materials.

Major Findings: It has been demonstrated on this contract that serum can be obtained directly from bovine fetuses with maintenance of sterility. The routine item of commerce is a material which has been filtered to remove bacterial contaminants and to which antibiotics are frequently added. The reagent has been distributed to a limited number of test recipients who are very active in cell culture research and virology. Any superiority of the unfiltered serum has not been clearly demonstrated.

Significance to Biomedical Research and the Program of the Institute: Fetal calf serum is an essential factor in the production of many viruses in cell culture systems. As the result of the availability of the unfiltered serum, biologic and biochemical standards can now be established for this reagent.

Proposed Course: The contract will be terminated on June 23, 1968.

Date Contract Initiated: June 24, 1966

Current Annual Level: June 24, 1967 - June 23, 1968: $61,200

MAKERERE UNIVERSITY COLLEGE (PH43-67-47)

Title: Epidemiologic Study of Burkitt's Lymphoma

Contractor's Project Director: Professor J.S.W. Lutwama

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: To conduct studies on the natural history, occurrence, and possible means of transmission of Burkitt lymphoma. To collect clinical specimens and follow-up data from patients with newly diagnosed and treated Burkitt lymphoma.

Major Findings: All major aspects of the contract are in operation with patients being identified, diagnosed, treated and followed. Specimens are being collected and shipped to NCI and field surveys are planned for large-scale incidence studies.

Significance to Biomedical Research and the Program of the Institute: This program is designed to answer many questions about the natural history of this acute lymphomatous disease thought to be of viral origin. In addition clinical specimens and biopsies provide basic material for laboratory studies conducted within the Special Virus-Leukemia Program.

Proposed Course: Work on this contract will proceed as in the past.

Date Contract Initiated: September 26, 1966

Current Annual Level: September 26, 1967 - September 25, 1968: $49,325

MARQUETTE UNIVERSITY (PH43-64-52)

Title: Human Embryonic and Fetal Tissue Procurement

Contractor's Project Director: Dr. Richard F. Mattingly

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: Procurement and shipping of aseptically obtained trophoblastic, embryonic, or perinatal tissue for distribution to cancer-virus research programs designated by the National Cancer Institute. Characterization of trophoblastic tissue employing functional and cytologic expression of endocrine activity as indicators of viability correlated with cultivation of the tissues *in vitro*.

Methods Employed: In appropriate cases of ectopic pregnancy or perinatal death, trophoblastic, embryonic, or perinatal tissue is aseptically obtained, placed immediately into nutrient media, and shipped to designated laboratories. Bacteriology, histology, pathologic diagnoses, and clinical data are collected on each case. Tissue used for characterization is grown in culture and assayed histologically and hormonally.

Major Findings: The contractor has developed liaison with all of the hospitals in the Milwaukee area, both private and public, thus making available to this program a relatively large amount of tissue of this type. In 1967 tissue samples from 131 cases were procured and 216 specimens were distributed. Related research is being done on the malignant potentiality of the human trophoblast utilizing *in vitro* and *in vivo* systems.

Significance to Biomedical Research and the Program of the Institute: The contractor is one of 12 procurement centers which constitute the Tissue Procurement Program of the NCI, a program which procures, stores and distributes human cancer tissue as well as normal tissue to investigators in cancer research who are supported by NCI funds.

Proposed Course: To continue procurement and research operations as in the past.

Date Contract Initiated: September 19, 1963

Current Annual Level: June 1, 1967 - May 31, 1968: $70,523

MASSACHUSETTS GENERAL HOSPITAL (PH43-65-1034)

Title: Cryogenic Preservation of Platelets and Leukocytes

Contractor's Project Director: Dr. Charles Huggins

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: To develop procedures and reagents for the preservation and recovery of platelets and leukocytes from normal donors and from patients with various forms of leukemia.

Major Findings: Preservation of leukocytes by using low concentrations of protective agents combined with solute (glucose, fructose or sodium chloride) plus albumin or PVP permit leukocytes to be frozen slowly to -85°C with good survival. One specimen from a patient with CLL showed even better preservation than was obtained with normal leukocytes.

Further studies on separation techniques for leukocytes have been carried out using complex polymers. Methods and techniques for achieving satisfactory yields of cells seem to be developing but problems with optimal freezing procedures are still encountered.

Standardization of techniques for the collection and processing of platelets has been adopted and evaluation of the product is now underway.

Use of ethylene glycol as a cryophylactic agent in the preservation of erythrocytes for use as reagets has been highly successful.

Significance to Biomedical Research and the Program of the Institute: Methods for better quantitative and qualitative preservation of blood elements are critical needs for laboratory research programs using leukocytes for establishing cell cultures and functional preserved platelets are a continuing need in clinical supportive therapy.

Proposed Course: This contract will be terminated at the end of the contract period on January 31, 1969.

Date Contract Initiated: June 23, 1965

Current Annual Level: February 1, 1968 - January 31, 1969: $73,635

MEDICAL RESEARCH CONSULTANTS (PH43-67-1368)

Title: Testing of Human Tumors for Viability

Contractor's Project Director: Dr. Robert Rafajko

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: To test fresh and frozen human tumor tissue for viability in tissue culture and to find methods for the establishment of a bank of cells from these tissues.

Methods Employed: Fresh human tumors are grown in tissue culture by explant and trypsinization methods. Viability and type of cellular outgrowth is determined by various methods. New methods of viable freezing of these tumors are also tested. Primary and cultured tumor cells are stored for use within NCI Viral Oncology Programs.

Major Findings: Approximately 75 frozen human tumors have been tested for viability, of which approximately 20% showed growth in tissue culture. Freshly obtained human tumors are now under study for methods of cultivation and preservation.

Significance to Biomedical Research and the Program of the Institute: Primary cultures of human tumors are required for NCI Viral Oncology Programs as source materials for isolation of viruses and viral genomes. This contract will provide material and methodology for the proper culture and storage of this material.

Proposed Course: This contract will be terminated at the end of the current contract period in June 1968.

Date Contract Initiated: June 23, 1967

Current Annual Level: June 23, 1967 - June 22, 1968: $64,920

MELPAR, INC. (PH43-68-80)

Title: Human Perinatal Tissue Processing Facility

Contractor's Project Director: Dr. John E. Verna

Project Officer (NCI): Miss Mary K. Asdell

Objectives: To provide for the processing, storage and distribution of viable human perinatal frozen cell suspensions. To improve existing techniques and develop new approaches for the *in vitro* cultivation of human tissues, as well as to provide for their successful preservation by freezing procedures.

Methods Employed: Conventional methodology pertaining to cell culture.

Major Findings: Overall production performance has been quite satisfactory. One thousand, seven hundred and sixty-five vials of human perinatal kidney and lung have been processed and useful data has been obtained on improved ways of processing kidney tissue, and on the processing of other tissues such as primary lung and subcultured kidney and lung.

Significance to Biomedical Research and the Program of the Institute: The contractor is one of 12 procurement and processing centers which constitute the Tissue Procurement Program of the NCI, a program which procures, processes, stores and distributes human cancer tissue as well as normal tissue to investigators in cancer research who are supported by NCI funds.

Proposed Course: This contract will be terminated at the end of the contract period in September 1968.

Date Contract Initiated: May 25, 1965

Current Annual Level: October 1, 1967 - September 30, 1968: $65,825

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-67-700)

Title: Development of Virus Reagents

Contractor's Project Director: Dr. John C. Parker

Project Officers: Drs. Robert Holdenried (NCI) and Wallace P. Rowe (NIAID)

Objectives: To develop reagents and tests for the detection of murine and other laboratory rodent viruses, to apply these and other tools in the determination of the importance of the indigenous virus in experimental systems and to study means for their elimination.

Major Findings: Routine service--The diagnostic service continues to accumulate serological data on the prevalence and distribution of naturally occurring viruses in laboratory animals. Guinea pig and rabbit colonies were included for the first time. Approximately 45,000 individual serological tests were performed. A study of inactivated viral reagents demonstrated that they did not loose titer. These are now available for use.

Approximately 150 specimens were examined for virus by Mouse Antibody Production tests. Lymphocytic choriomeningitis virus (LCM) was detected in some mouse tumors used in cancer chemotherapy studies while other tumor lines were determined to be free of infection. Identified infected tumor lines can now be destroyed.

Significance to Biomedical Research and the Program of the Institute: The virus diagnostic capabilities provided the NCI with the ability to monitor laboratory rodent colonies and rodent produced virus reagents and tumors which resulted in production of highly characterized systems for cancer research use. Assistance and guidance provided under this contract was particularly important for the detection of LCM in rodent systems. This virus, in addition to being infectious for humans, has been difficult to diagnose.

Proposed Course: Continue with the expansion of the virus detection capabilities to viruses new to the Program.

Date Contract Initiated: April 10, 1961

Current Annual Level: March 1, 1968 - February 30, 1969: $368,245

MONTREAL CHILDREN'S HOSPITAL (PH43-65-1020)

Title: Supply of Leukemic and Normal Blood Plasma Specimens

Contractor's Project Director: Dr. Ronald L. Denton

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: To obtain plasma specimens from pediatric leukemic patients and nonleukemic controls, storing and making them available for a wide variety of research purposes.

Major Findings: During the period, plasma or serum samples were procured from 8 untreated pediatric acute leukemic patients, 24 control patients and 50 follow-up specimens were obtained for a total of 82 cases. Twenty-three specimens were shipped to research laboratories.

Significance to Biomedical Research and the Program of the Institute: This is a resource contract for the supply of plasma and serum specimens from leukemic and nonleukemic children for the study by scientists participating in the Special Virus-Leukemia Program in a search for viruses associated with human leukemia.

Proposed Course: Continue to collect serum specimens.

Date Contract Initiated: September 24, 1965

Current Annual Level: June 24, 1967 - May 31, 1968: $10,845

PADUA, CITY HOSPITAL OF (PH43-66-892)

Title: Collection of Human Mammary Adenocarcinoma Specimens

Contractor's Project Director: Professor Giovanni Dogo

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: To procure and ship human mammary adenocarcinoma and other large primary tumors to NCI for use in research programs.

Methods Employed: Under the supervision of the responsible surgeon, surgical residents will collect tumor tissue at the operating table, and perform preliminary processing prior to shipping it in a frozen state to Bethesda. Hospitals at which materials will be collected are within a 50-mile radius.

Major Findings: To date 70 samples of breast carcinomas, of over 20 grams each, have been shipped.

Significance to Biomedical Research and the Program of the Institute: Human tumors for tissue culture study are needed within the research programs in viral oncology. Such material will be necessary for the isolation of viruses and viral genomes.

Proposed Course: To continue procurement operations as in the past.

Date Contract Initiated: October 27, 1964

Current Annual Level: June 7, 1967 - June 6, 1968: $8,338

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (PH43-66-950)

Title: Production of Simian Viruses and Homologous Antiserum

Contractor's Project Director: Dr. S.S. Kalter

Project Officer (NCI): Dr. James T. Duff

Objectives: To prepare and test small pools (300-500 ml) of virus and rabbit antiserum for selected simian viruses. To test and/or certify simian virus reagents submitted to contractor by NCI for this purpose.

Methods Employed: Virological procedures in production and testing of viruses and antisera.

Major Findings: Reagents (seed virus and antiserum) are being prepared for a variety of simian viruses, including SV42-49, SA5, SA6, SA10-13, SA17, SA18 and V340. Other simian viruses will be added to this list as time permits. With the exception of SA10, SA11 and SA13, virus pools have been prepared for the remaining virus preparations and testing of antisera against these viruses is in progress. The viruses designated by the SA prefix are from South Africa and have not been readily available to scientists in this country. Additional characterization studies on these viruses are in progress.

Significance to Biomedical Research and the Program of the Institute: NCI has made a justifiable commitment to the use of subhuman primates to determine whether animals phylogenetically close to man will serve as sensitive indicators of viral replication and of specific disease induction capability of potential cancer viruses and chemical carcinogens. When neoplastic diseases occur or are induced in subhuman primates, it will be essential to know what contribution, if any, was made by indigenous viruses carried by the recipient primates. These reagents will be made available to the various NCI intramural and collaborating scientists working with nonhuman primates.

Proposed Course: Preparation of simian virus reagents will be completed during the current contract year. Simian virus reagents prepared by this contractor and other contractors will be packaged for distribution by the Ben Venue Laboratories (PH43-68-633). The final packaged materials will be submitted to SWFRE for testing and/or certification prior to being distributed by NCI. SWFRE will develop procedures for the use of these reagents by other investigators in preparing working reagents of their own, standardization of working reagents, and performing identification of unknown simian virus agents. These protocols would be available to persons requesting the reagents from NCI.

Date Contract Initiated: June 15, 1966

Current Annual Level: February 1, 1968 - January 31, 1969: $55,625

TULANE UNIVERSITY (PH43-64-881)

Title: Tree Shrews for Cancer Research

Contractor's Project Director: Dr. Norman Negus

Project Officers (NCI): Drs. Roy Kinard and Robert Holdenried

Objectives: Establish a breeding stock of at least one species of tree shrew and conduct and encourage cooperative studies to establish and improve the utility of this animal for cancer research.

Methods Employed: Special animal husbandry is being developed to establish a productive breeding system. Information gained is made available to prospective users of tree shrews in cancer research.

Major Findings: One animal developed a spontaneous sarcoma. Cell cultures of this sarcoma are being studied for viral susceptibility at the virus laboratory of the Baylor University under Contract PH43-68-678. Breeding has been moderately successful in the tree shrew colony.

Significance to Biomedical Research and the Program of the Institute: The tree shrew, a primative primate or closely related to the primative primates, may be, as a result of basic evolutionary position, susceptible to viruses of rodents and primates. It is also a small animal producing up to 3 young at one time with a short gestation period, all of which are characteristics that make it a potentially economical laboratory animal.

Proposed Course: Produce largest possible number of young, develop means of raising young separated from their mother, encourage their use to determine suitability for laboratory work, and extend virus studies that are underway.

Date Contract Initiated: June 3, 1964

Current Annual Level: March 1, 1968 - February 30, 1969: $19,257

TULANE UNIVERSITY (PH43-64-927)

Title: Human Perinatal Tissue Procurement Center

Contractor's Project Director: Dr. Charles E. Dunlap

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: To provide viable human perinatal tissue to the Tissue Procurement Program for distribution to cancer-virus laboratories operated by or affiliated with NIH.

Methods Employed: Kidneys are removed aseptically from recently deceased perinatal cadavers and processed to obtain vials of frozen viable cell suspensions which are stored at liquid nitrogen temperature until distribution. Other organs and tissues are also procured and processed. Quality control testing and characterization studies include microbiological screening viability assay by cell culture, and examination of genetic parameters by both morphologic and biochemical techniques.

Major Findings: In 1967 tissue was obtained from 83 cases. Perinatal lung, thymus, trachea, and thyroid are also regularly supplied.

Significance to Biomedical Research and the Program of the Institute: The contractor is one of 12 procurement centers which constitute the Tissue Procurement Program of the NCI, and program which procures, stores, and distributes human cancer tissue as well as normal tissue to investigators in cancer research who are supported by NCI funds.

Proposed Course: This contract will be terminated at the end of the contract period in October 1968.

Date Contract Initiated: June 30, 1964

Current Annual Level: June 1, 1967 - October 31, 1968: $96,216

UNIVERSITY OF CALIFORNIA (PH43-63-13)

Title: Certification and Standardization of Mammalian Cell Cultures

Contractor's Project Director: Dr. Stewart H. Madin

Project Officer (NCI): Dr. James T. Duff

Objectives: To develop and evaluate selected cell lines for use as substrates for growth of cancer viruses.

Methods Employed: Cell culture and virological methods.

Major Findings: Several new cell lines of cat and canine origin have been established and are available as trial substrates for propagation of cancer viruses isolated from these species. Collaborative studies on feline leukemia are in progress with Dr. Theilen, University of California, Davis, California. Canine cell lines are being sent to Dr. Gilden, Flow Laboratories, Inc., for studies on canine leukemia. At the suggestion of Dr. Huebner, work is in progress on the establishment and preservation of normal parakeet cell lines which will be used in attempts to recover tumor viruses from this species. Additional collaborative research on bovine leukemia with Dr. Theilen on various cell cultures derived from tissues of leukemic cattle is continuing. Several cultures of thymus cells and cells of reticuloendothelial origin have been established from a heifer with clinically confirmed lymphosarcoma.

Significance to Biomedical Research and the Program of the Institute: This contractor has been the major source for the development of cell lines from the major groups of non-primate mammals. Many of these cultures have been dispensed through the American Type Culture Collection for a wide variety of studies by the scientific community and to investigators conducting specific studies related to NCI's program.

Proposed Course: Since 1962 the cell culture development and characterization studies conducted by NBL have been closely associated with the Cell Certification Program of the American Type Culture Collection. These studies are now being phased out. The major effort now involves research and development of cell substrates of immediate usefulness to NCI's Viral Oncology Program. Currently, major emphasis is on the development of cell lines from the dog, cat, cow and parakeet. These cell strains will be made available to contractors in the Special Animal Leukemia Ecology Studies Segment and the Solid Tumor-Virus Segment.

Date Contract Initiated: October 1, 1967

Current Annual Level: October 1, 1967 - September 30, 1968: $176,220

UNIVERSITY OF CONNECTICUT (PH43-62-505)

Title: Development and Maintenance of a Specific Pathogen Free Flock of White Leghorn Chickens

Contractor's Project Director: Dr. Roy E. Luginbuhl

Project Officers (NCI): Drs. Robert Holdenried and Roy Kinard

Objectives: Establish and maintain a flock of chickens free of specified pathogens including avian leukosis viruses and to provide a limited number of eggs for research use.

Methods Employed: Special animal husbandry methods include development of subflocks maintained in small individually separated houses.

Major Findings: At least a portion of the flock, established in former contract years, has been maintained free of disease. Approximately 14,000 eggs were provided to cancer research. Marek's disease continues to be most difficult to exclude.

Significance to Biomedical Research and the Program of the Institute: The methods being developed indicate that eggs free of specified infectious organisms can be produced. A significant portion of the avian leukosis research in the United States is dependent on the continued availability of this highly controlled and monitored flock.

Proposed Course: Continued maintenance of the flock with development of genetic lines of chickens characterized for the susceptibility of their embryos to leukosis virus. Methods for more complete isolation of units of the flock will be investigated.

Date Contract Initiated: June 18, 1962

Current Annual Level: November 1, 1968 - October 30, 1969: $60,000

UNIVERSITY OF IBADAN (PH43-67-674)

Title: Procurement and Culture of Burkitt Lymphoma Clinical Materials

Contractor's Project Director: Professor G.M. Edington

Project Officer (NCI): Dr.Robert H. Depue, Jr.

Objectives: To obtain serum specimens and biopsies from patients suffering from Burkitt lymphoma and to attempt to establish cell lines from this tumor in tissue culture. These materials will be shipped to NCI.

Methods Employed: Patients are located and brought to the hospital for treatment. Serum specimens and biopsies are obtained before treatment. Follow-up specimens are obtained from patients after treatment and at appropriate intervals. Successful tissue cultures and serum specimens are shipped to NCI for use within the Special Virus-Leukemia Program.

Major Findings: Six biopsies have been cultured and four cultures are still viable, although it is too early to be certain of a long term continuous propagation. Serum specimens have been obtained from 15 patients.

Significance to Biomedical Research and the Program of the Institute: Tissue specimens and serum from patients with Burkitt lymphoma are necessary to determine the relationship of the herpes-like virus present in this tumor to this disease and to infectious mononucleosis. This virus is the first candidate cancer virus isolated within SVLP.

Proposed Course: The continuation of this project is subject to approval by the Office of the Surgeon General. Request for continuation has been submitted to that Office but no approval has been received.

Date Contract Initiated: April 13, 1967

Current Annual Level: April 13, 1967 - April 12, 1968: $51,395

UNIVERSITY LABORATORIES, INC. (PH43-66-1133)

Title: Production of Sarcoma Viruses and Their Antisera

Contractor's Project Director: Dr. Eugene H. Bernstein

Project Officers (NCI): Drs. Robert Holdenried and Harry G. Steinman

Objectives: Production of sarcoma viruses and antisera.

Methods Employed: Partially purified virus is harvested from mouse tumors following intramuscular inoculation. Purification procedures include differential centrifugation.

Major Findings: Hepatitis contamination forced cessation of Moloney sarcoma virus and antisera production for 2 months until disinfection was realized. Production resumed January 1, 1968, with mice from a new source. Seed RAV virus contained a bacterial contaminant now eliminated by filtering. Reo 3 virus contamination was reported in mice from the new source. Virus lots of MSV 6, 7, and 8 were produced prior to general disinfection, but are also suspected to have hepatitis virus contamination. Since January 1968 Moloney sarcoma virus production has been at an accelerated rate and should meet contract required amounts by September 1968. RAV-1 has been produced.

Significance to Biomedical Research and the Program of the Institute: Moloney sarcoma virus and RAV viruses of standard virulence and known freedom from contaminants are available for research use. Each research laboratory may obtain a well defined standard virus preparation which should lead to readily reproducible results.

Proposed Course: Increase rate of Moloney sarcoma virus production to compensate for the 2 months loss of production. Tissue culture production of Moloney and Rauscher pseudotype viruses. Continue production of RSV (RAV-2). Produce RSV (RAV-2) antisera in chickens. Characterize RSV (RAV-2) in chicken wing web and in quail and hamsters intracerebrally.

Date Contract Initiated: June 4, 1962

Current Annual Level: October 1, 1967 - September 9, 1968: $184,780

UNIVERSITY OF LOUISVILLE (PH43-66-902)

Title: Preparation of Simian Foamy Virus Reagents and Antisera

Contractor's Project Director: Dr. Paul B. Johnston

Project Officer (NCI): Dr. James T. Duff

Objectives: To prepare and test reference reagents (seed material and antiserum) for simian foamy viruses Types 1-7.

Methods Employed: Virological procedures.

Major Findings: Virus seed pools and antisera were prepared and submitted to NCI for simian foamy agents Types 1-4. The Types 1 and 2 reagents were satisfactory and are available for packaging. The Type 3 virus seed pool and antisera were found to be contaminated with Type 1 virus and were re-made. The Type 4 virus was contaminated with an "enterovirus agent" presumably from the rabbit kidney used to grow the virus. A new virus pool for Type 4 virus was prepared. Virus pools for Types 6 and 7 have been prepared and rabbits have been immunized against these viruses. Preparation of seed material for Type 5 agent is in progress. These reagents should be available at NCI by late June 1968.

Significance to Biomedical Research and the Program of the Institute: The simian foamy virus reagents will be useful in identifying unknown viruses and antibodies in non-human primates used for the study of the viral etiology of cancer so that a more careful assessment can be made of any viruses isolated from an induced or spontaneous neoplasm. The sera may also be useful in suppressing the growth of these viruses as adventitious agents in culture of simian tissues.

Proposed Course: Complete preparation and testing of reagents. Contract will be extended (without additional funding) to enable contractor to complete essential testing of reagents.

Date Contract Initiated: June 13, 1966

Current Annual Level: June 13, 1967 - June 30, 1968: $17,105

UNIVERSITY OF NOTRE DAME (PH43-65-1018)

Title: Development and Application of a Virus-free Laboratory Mouse for Leukemia Research

Contractor's Project Director: Dr. Morris Pollard

Project Officers (NCI): Drs. Robert Holdenried and A.J. Dalton

Objectives: This contract calls for the identification of a laboratory mouse strain free of virus-like particles. Identified virus-free strains will be maintained germfree. The biological activity of C-type particles found in "germfree" mouse stocks, natural occurring and induced leukemia will be studied.

Major Findings: With the range of mouse strains examined, it is now quite clear that there is probably no strain free of leukemia virus. The Lobund germfree rats, however, seem to lack particles. Yet, when treated with chemical carcinogens, they develop leukemia.

Significance to Biomedical Research and the Program of the Institute: No leukemia free strain of mice is available for cancer research. This fact must be considered when using mice in cancer research.

Proposed Course: With the finding that all strains of laboratory mice are infected with leukemia virus, the aim of the project became untenable. The project will be terminated with receipt of the final report at the end of April 1968.

Date Contract Initiated: June 10, 1965

Current Annual Level: $48,325 for 11 months

UNIVERSITY OF TEXAS, M.D. ANDERSON HOSPITAL (PH43-68-631)

Title: Procurement of serum and tumors from cancer cases.

Contractor's Project Director: Dr. Carl F. Tessmer

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: To collect serum samples and tumors at surgery from individuals with various malignancies and to ship these specimens as directed by the Project Officer.

Methods Employed: Tumors are collected at surgery along with appropriate clinical information. The tissue is placed in culture media and shipped to recipients as directed by the Project Officer.

Major Findings: Shipment of specimens commenced in February and will continue at a rate of 2 to 3 a week.

Significance to Biomedical Research and the Program of the Institute: Human tumors for tissue culture study are needed within the research programs in Viral Oncology. Such material will be necessary for the isolation of viruses and viral genomes.

Proposed Course: The contract will continue to provide human tumors for study.

Date Contract Initiated: November 8, 1967

Current Annual Level: November 8, 1967 - November 7, 1968: $32,485

ZOOLOGICAL SOCIETY OF SAN DIEGO (PH43-63-56)

Title: Experimental Breeding of Nonhuman Primates

Contractor's Project Director: Dr. Robert W. Cooper

Project Officers (NCI): Drs. Robert Holdenried and Roy Kinard

Objectives: To determine if selected small primate species can be successfully bred in captivity under the conditions provided, to develop nucleus breeding stocks reasonably free of pathogens, to determine nutritional requirements, and growth characteristics.

Methods Employed: Special animal husbandry procedures adapted to each species continue to be developed.

Major Findings: In addition to supplying limited numbers of newborn animals to several research projects, primarily to the Bionetics Research Laboratories which operate under another SVLP contract, newborn galagoes have been, in collaboration with the Laboratory of Chemical Pharmacology, NCI, inoculated with chemical carcinogens at San Diego. At least one developed a maliganant tumor. After a couple of years' failure in attempts to purchase adult talapoins (a very small species of the African green monkey complex) the Project Director, together with an established animal importer, went to Africa to obtain a number of these animals. They were successful and about 40 talapoins are now (since March 1968) in the colony at San Diego, and a commercial import source is being developed.

Significance to Biomedical Research and the Program of the Institute: The species of primates in addition to rhesus and baboons available for research are being expanded.

Proposed Course: The species under study will be continually evaluated. Those found unresponsive to reproduction in the colony will be eliminated and new species introduced as required.

Date Contract Initiated: August 8, 1962

Current Annual Level: March 1, 1968 - February 30, 1969: $120,840

SPECIAL ANIMAL LEUKEMIA ECOLOGY SEGMENT

Dr. John B. Moloney, VLLB, Etiology Area, Chairman
Dr. Michael B. Chirigos, VLLB, Etiology Area, Vice Chairman

UNIVERSITY OF PENNSYLVANIA (PH43-65-1013)

Title: Research in Experimental and Natural Transmission of Bovine Leukemia

Contractor's Project Director: Dr. Robert Marshak

Project Officer (NCI): Dr. John B. Moloney

Objectives: To describe the etiology of bovine leukemia. To attempt experimental transmission of leukemia through the use of cell-free materials prepared from selected leukemic milks and solid tissue. To attempt natural transmission of the disease through reciprocal foster nursing experiments.

Major Findings: Studies on early diagnosis of leukemia indicate that aneuploidy of lymphoid cells is probably a good criterion of early disease. Biopsy of subcutaneous hemal lymph nodes appears to be a useful procedure in early diagnosis. They have become proficient in preparing thoracic duct-venous shunts for long-term collection of thoracic duct lymphocytes from normal and leukemic cows. Twelve cell lines from 4 cows with leukemia have been established in continuous culture (stationary or spinner) for periods of between 5 and 10 months. These are aneuploid, mononuclear cells. Preliminary EM studies have failed to demonstrate VLP.

Viral interference studies have shown that resistance is a common finding in bovine cell cultures from many sources and probably does not have much value in detection of a bovine leukemia virus. VLP resembling murine leukemia virus have been detected in tissues, cell cultures, and milk derived from leukemic and normal cattle. Cow's milk appears to be richest source of these VLP. Inoculation of bovine tissue extracts, cell culture material and/or milk concentrates containing VLP into rats, hamsters, mice, and cell cultures have been unsuccessful (no leukemias or replication of VLP). Studies have been initiated to attempt rescue of Moloney sarcoma virus genome with VLP containing bovine leukemia specimens. The reciprocal foster nursing experiment, designed to clarify the relationship between ingestion of milk and occurrence of lymphocytosis and leukemia, is well underway.

In attempts to improve success rate in transplantation experiments they have begun evaluating the use of horse-anti-cow lymphocyte serum as an immunosuppressive agent. Attempts to transmit leukemia with leukemic thoracic duct lymph and lymphocytes, and with 12 established bovine leukemia cell lines are underway in newborn calves from defined herds. Attempts to transmit leukemia by injection of cell-free materials (e.g. virus-like milk particles (VLP) cell fractions from cell lines, and leukemic lymph nodes, thymus and spleen) are also underway in defined newborn calves. Leukemia induction experiments with 7,12-DMBA in calves have been completed and animals are being observed for development of the disease.

UNIVERSITY OF PENNSYLVANIA (PH43-65-1013)

Significance to Biomedical Research and the Program of the Institute: This study has been one of a series of collaborative studies with Dr. Dutcher, Institute for Medical Research, and Dr. Burger, State University of New York. Amalgamation of Dr. Dutcher's research group with the U. of Pa. group took place on Feb.1, 1968. With such collaborative efforts it is believed that the bovine leukemia problem can move forward at a more rapid pace. This contract study is an integral part of the SALES program on bovine leukemia.

Proposed Course: Reciprocal foster nursing experiments will continue with the introduction of several new genetically and hematologically defined nurse cows and calves from such defined dams. Continuing attempts will be made to improve success rate in transplantation experiments (especially through use of horse-anti-cow lymphocyte serum). Transplantation experiments which fail, most particularly those in which transplanted tumors develop and regress, are also worthwhile because of the possibility that a virus-induced tumor may develop in some recipients after an appropriate latent period. Attempts to transmit leukemia by injection of leukemic thoracic duct lymph and lymphocytes and our leukemia cell lines will continue. Attempts to transmit leukemia by injection of various cell fractions from leukemic lymph node, spleen, thymus and leukemia cell lines and by injection of VLP milk concentrates will continue. Only newborn calves from defined herds in which matched controls (genetic background, age, etc.) are available will be used in these experiments. A minimum of 20 animals per experimental group will be used. Animals will be monitored by EM, clinical, hematological, cytogenetic, and immunological techniques as indicated.

Animals in 7,12-DMBA experiments will be observed for development of leukemia. No new DMBA studies will be undertaken until results of present experiments can be assessed. Studies on early (pre-clinical) diagnosis of leukemia will continue Attempts to demonstrate VLP in cell lines by EM and immunoflourescence will continue. Cells established in continuous culture will also be studied to determine whether or not they are producing immunoglobulins. Cell lines will be studied to determine optimum culture conditions and will also be frozen away. They will continue to be propagated in large volumes from transmission experiments.

The following will be also continued: Methods for the extraction and purification of virus-like particles from cow's milk, plasma, tissue, and tissue cultures, and establishment of their biological, chemical and physical nature; development of detection and identification systems for bovine leukemia, utilizing electron microscopy, cell culture and immunologic techniques; collaboration with investigators at other institutions.

Date Contract Initiated: 6/18/65

Current Contract Level: $572,485 (Feb. 1, 1968 - Dec. 31, 1968)

MICHIGAN STATE UNIVERSITY (PH43-65-100)

Title: Leukemia Transmission Studies in the Dog

Contractor's Project Director: Dr. Gabel H. Conner

Project Officer (NCI): Dr. George J. Burton

Objectives: To define the etiology of canine leukemia; to relate these findings to the problem of leukemia in humans. To determine if an association exists between canine and human leukemia.

Major Findings: Results of inoculations of 329 neonatal beagle dogs with candidate leukemogenic materials have been: (a) 3 malignant lymphoma deaths occurred from whole canine lymphoma cells and 1 from extract in less than 90 days; (b) mast cell leukemia is induced only with whole mast cells, in 47 to 70 days; (c) inocula of human or avian origin have yielded negative results; (d) one puppy (60001) inoculated with rat sarcoma virus had reticulum cell type malignant lymphoma 4 months later, with regression to hyperplasia 13 months subsequently and at present. One hundred and twenty-seven inoculated older dogs ranging in age from 9 months to 25 months, are being held at the isolated, long-term holding facility, the H-Bar-B Kennels in Essexville, Michigan, about 75 miles northeast of Lansing.

The viral findings have been: (1) isolation of herpes-like MDH-1 virus in canine thymus cells that had been inoculated with P3-J Burkitt cells superinfected with Moloney sarcoma virus; (2) isolation of a canine herpesvirus from a ML lymph node; (3) demonstration of herpes-like particles in 21 (23.3%) out of 90 dogs with spontaneous ML; (4) demonstration of C-type particles *in vitro* in transformed dog ML cells; (5) demonstration of C-type particles *in vitro* from a lymph node of induced canine mast cell leukemia; (6) demonstration of budding virus particles in a cell line derived from a spontaneous canine fibrosarcoma; (7) demonstration of a few C-type particles in 3 cases of ML; (8) demonstration of adeno-like viruses in tonsils of 5 out of 25 street dogs; and (9) demonstration of crystalline arrays, considered as virus-associated, in EM preparations made from most dogs having ML, and from some dogs with distemper. With the exception of the MDH-1 virus, all of these particles are of canine origin.

MDH-1 virus causes a herpes-like CPE in canine kidney cells *in vitro*, but also has the same effect on human and bovine kidney cells. Canine thymus cells inoculated with MDH-1 became transformed into a suspended cell culture of the Burkitt type. Puppies, immature Rhesus monkeys, mice, and hamsters were not affected by inocula of MDH-1. The exact nature and role of MDH-1 virus remains to be clarified.

Basic pathological and hematological studies have been completed on 60 dogs with spontaneous malignant lymphoma. Sixty-four per cent were found to be reticulum-cell type, and 28 per cent were the lymphocytic type. The hematological studies have yielded no unusual findings, after comparison with the normal range of the various types of leucocytes in dogs.

MICHIGAN STATE UNIVERSITY (PH43-65-100)

The sera from 34 dogs with malignant lymphoma were examined by immunoelectrophoresis for evidence of protein abnormalities. Seventeen of the dogs were deficient in Immunoglobulin G, and 12 in Immunoglobulin A. Nine dogs had a slight increase in IgA. Increased haptoglobin levels were found in 22 dogs with lymphoma. Two of 10 rabbit sera from rabbits immunized with dog lymphoma extracts contained complement-fixing antibody (1:128 and 1:32) against herpes simplex virus; one of the two sera also contained CF antibody against human adenovirus (1:32).

Twenty-four isolator-reared puppies were irradiated to determine effect of varying doses. The most significant finding was that a single 200 r dose resulted in depression of immune capabilities as determined by leucocytic indices. A dose of 225-250 r would most likely totally suppress immune capabilities.

Significance to Biomedical Research and the Program of the Institute: The association of dogs with humans is probably closer than that between any other domestic animal and man. The fact that a clarification is needed of the transmission of lymphosarcoma in the dog, as well as the transmission of leukemia and lymphosarcoma in man, indicates the need for intense investigations of the former in relation to the latter, and the search for a possible common transmission agent in view of similarities between the two diseases. This type of attack, therefore, is similar to that upon any other disease which may involve both humans and animals in the life cycle of the causative agent. By isolating, identifying, and studying viruses found in both dog and human leukemia, similarities may emerge which may link one disease with the other, especially in view of the onset of lymphosarcoma in some dogs which have been in contact with leukemic persons.

Proposed Course: In vitro techniques will be continued in order to facilitate the isolation and/or propagation of an oncogenic agent in the infective state in sufficient quantity to afford a more consistent induction of malignant lymphoma in experimental hosts. If unproductive, studies on the MDH-1 virus may be discontinued. Emphasis will also be placed on fewer inocula in more dogs. Inoculations will be initiated into another breed of dog having known natural spontaneous lymphoma in adequate numbers. Cell-free inocula of C-type particles from cases of canine spontaneous lymphoma and from cell lines will be stressed.

Date Contract Initiated: April 4, 1965

Current Contract Level: $615,830

UNIVERSITY OF MINNESOTA (PH43-65-606)

Title: Research on Susceptibility of Cows to Known Tumor Viruses

Contractor's Project Director: Dr. Dale Sorensen

Project Officers (NCI): Dr. Michael A. Chirigos, Dr. Mearl Stanton, Dr. William A. Hook

Objectives: To determine the susceptibility of cattle to murine leukemia viruses (Moloney and Rauscher).

Major Findings:

1. Five calves were inoculated with either murine leukemia virus or a placebo on a random basis and now all 50 calves have been inoculated. Monitoring studies of the 30 calves inoculated with murine leukemia viruses and the 20 control calves were continued and have revealed no evidence of leukemia. This study includes clinical examination, hematologic evaluation, serum neutralization tests (SN), complement fixation tests (CF), and virus isolation tests. The serologic tests (SN and CF) have revealed that antibodies have been produced against the murine viruses. No virus isolation from the inoculated calves has been made by mouse inoculation.

2. Five calves were inoculated with Moloney rhabdomyosarcoma virus and other possible helper viruses. No signs of tumor growth have been observed.

3. Twelve intrauterine fetal inoculations using Moloney or Rauscher virus were performed. It appears that 7 of these were successful and the fetuses are still alive and developing. Attempts to isolate virus from the fetuses which reabsorbed were not successful.

4. Bovine endocardial cells exposed to murine leukemia virus failed to show any typical "C" type particles on electron microscopic studies performed 2, 3 or 4 weeks after virus exposure.

5. Electron microscopic studies of lymphocytes from BALB/c mice inoculated with Rauscher virus revealed many extrusions of the outer component of the nuclear envelope.

Significance to Biomedical Research and the Program of the Institute: A relation of bovine leukemia to human leukemia has not been demonstrated. However, the occurrence of the disease in cattle producing milk for children's consumption is of public health concern. Until a bovine leukemia virus is available for research use, significant leading information may be obtained from the study of murine leukemia virus in dairy cattle.

UNIVERSITY OF MINNESOTA (PH43-65-606)

Proposed Course: This program had been operating for two and one half years and there had been essentially negative results, therefore it was decided to terminate the contract on December 31, 1967, especially since the SALES program has two other bovine leukemia research contracts.

Date Contract Initiated: 6/21/65

Current Contract Level: June 21, 1967 - December 31, 1967: $82,590

UNIVERSITY OF NORTH DAKOTA (PH43-66-8)

Title: Insects, Ticks and Mites as Possible Experimental and Natural Vectors of Murine and Avian Tumor Viruses.

Contractor's Project Director: Dr. Robert G. Fischer

Project Officer (NCI): Dr. George J. Burton

Objectives: To determine the role, if any, that arthropods may play in transmission of tumor viruses, particularly those of murine and avian origin.

Major Findings: The viability of four tumor viruses (3 murine and 1 avian, was determined in 2 species of conenose bugs, 1 species of mosquito and 1 species of soft tick. Results were as follows:

Tumor Virus	Arthropod Species	Virus Viability in Days
Rauscher murine leukemia virus	*Triatoma infestans* (conenose bug)	15 days
	T. infestans hemolymph	3 days
	T. infestans feces	1 day
	Rhodnius prolixus (conenose bug)	26 days
	Aedes aegypti (mosquito)	0 days
Friend murine leukemia virus	*Triatoma infestans*	20 days
	Rhodnius prolixus	1 day
	Aedes aegypti	1 day
Moloney sarcoma virus	*Triatoma infestans*	4 days
	Rhodnius prolixus	6 days
	Aedes aegypti	0 days
	Aedes aegypti eggs	0 days
	Aedes aegypti larvae	0 days
	Alphitobius diaperinus (beetle)	1 day
Avian reticulo-endotheliosis virus (Twiehaus strain)	*Triatoma infestans*	3 days
	Rhodnius prolixus	12 days
	Argas persicus (soft tick)	6 days

These results indicate that the vector, Aedes aegypti cannot maintain any of the leukemogenic viruses tested, and that conenose bugs are good incubators of tumor viruses.

Studies were carried out on attempted mechanical transmission by interrupted feeding. All experiments to date with all viruses and all the arthropods listed here have been negative.

All biological transmission experiments following the natural course of feeding, i.e. a full blood meal on a viremic mouse or chick, then a subsequent full blood meal on a healthy mouse or chick, have been negative, except for one experiment involving Friend virus as follows: Five cages of 5 Triatoma infestans were fed to completion of Friend leukemia infected mice, were then maintained for 20 days in an environmental chamber, and then allowed to feed again on healthy mice. Three out of 4 of the latter mice developed the myeloproliferative form of the disease. Electron microscopic study would be valuable in determination of the presence and number of viral particles within various organs of this species, following infection by feeding on a viremic mouse.

Significance to Biomedical Research and the Program of the Institute: Arthropods have been investigated in relation to the transmission of human diseases for many years. Viruses, which are obligate parasites, may be transferred by arthropod vectors, and such transmission may occur biologically, mechanically, or through secretion or excretion of the vector. Arthropods such as mosquitoes, bedbugs, biting flies, fleas, conenose bugs, ticks and mites may suck blood from both man and from domestic animals (including birds) closely associated with man; therefore, it is important to investigate whether leukemogenic viruses present in infected animals can be transferred to humans. If such vector or vectors are found as a result of the investigations under this contract then measures can be taken to control the particular vector, and to prevent contact between vector and human or animal hosts, and thus to prevent transmission of the particular leukemogenic virus to man.

Proposed Course: As additional colonies of insects are acquired and developed, these studies will include research on additional mosquitoes, bedbugs, biting flies, etc. Greater emphasis will be placed on "natural" transmission experiments. EM studies to detect CPE and multiplication of tumor viruses in arthropods will be initiated as necessary.

Date Contract Initiated: 10/27/65

Current Contract Level: $112,000

UNIVERSITY OF CALIFORNIA (PH43-65-609)

Title: Bovine Leukemia Transmission Studies

Contractor's Project Director: Dr. Gordon H. Theilen

Project Officer (NCI): Dr. John B. Moloney

Objectives: To attempt experimental and natural transmission of bovine leukemia using selected cell-free concentrates of milk and solid leukemic tissue. To define the etiology of bovine leukemia.

Major Findings: Many thousands of gram equivalents have been concentrated from tissue of 18 cows and from plasma of 23 cows. This, plus additional concentrated material, will make up a pool of concentrated tumor tissue that will be inoculated into calves. Six calves are alive and inoculated with large quantities (250 to 1,000 gram equivalents) of cell-free tumor extracts.

Viral-like particles have been found in plasma of two leukemic and two inoculated cows. These particles have not been found in plasma of four normal cows. They closely resemble but are not identical to type "C" virus, have double outer limiting membranes, and a somewhat dense nucleoid. They are found in sucrose density gradient bands in the region of 1.15 to 1.17 gm/cm^3, but not in malignant and normal tissue of five cows with leukemia.As regards cell culture, another bovine leukemia cell suspension culture (BL-2) has been developed. It also is a lymphoid cell and maintains malignant cell characteristics. Twenty newborn hamsters, 25 newborn mice, and 2 calves with BL-1 suspension cells have been inoculated. The calves developed cytotoxic antibodies against BL-1 cells. Persistent lymphocytosis is present in 43% of the inoculated cattle. A few of these cows have chromosome aberrations such as aneuploidy, endoreduplication, and translocation. Persistent lymphocytosis (PL) can be produced in calves by inoculation of leukemic whole cells or buffy coat cells with or without the addition of bone marrow. PL was transferred through 4 serial passages, but in the fifth passage none of the animals have developed a lymphocytosis. None of the animals in this experiment have yet developed lymphosarcoma; however, several have extreme blood dyscrasias mimicking preleukemic lesions observed in the past. The significance of this so called "pre-leukemic" change is still not completely known.

EM studies of peripheral blood lymphocytes from PL cattle (spontaneous and induced) and leukemics have demonstrated a number of cells with a nuclear outpocketing. This nuclear abnormality also was found in BL-1 cells. Cytogenetic studies of the animals in serial whole cell transfer have been completed. There was not a consistent structural change which could be associated with persistent lymphocytosis. However, in some animals the number of cells with polyploidy was increased above what is expected in normal cells.

Significance to Biomedical Research and the Program of the Institute: The nature of bovine leukemia suggests a possible viral etiology; the findings of leukemia-like particles (type C) in milk and tissue of the bovine further this suggestion. Studies into the etiology and transmission of bovine leukemia by cell-free preparations (virus materials) is of importance from a purely economic aspect, plus the possibility of the transmission of leukemia from domestic animals to man. The almost constant exposure of man to bovine products warrants continuation of efforts in this direction, directed toward a more complete understanding into the nature and cause of bovine leukemia, and the relationship of this disease to leukemia as it occurs in man.

Proposed Course: The necessity to enhance the biological potency of the presumed virus before *in vitro* studies will be pursued productively. It will be necessary to delineate more precisely the relationship between persistent lymphocytosis and leukemia.

Transmission of leukemia in cattle using cell-free and cellular inocula will be continued. Discussions have been held concerning possible methods of indicating that persistent lymphocytosis (PL) is a pre-leukemic state, which would be a more satisfactory way of detecting infection than by waiting several years for leukemia to develop in some of the affected cows. These methods will include demonstration of PL as a possible immuno-proliferative disorder, and attempts to precipitate leukemia by physical and/or chemical means.

More sensitive biophysical methods of detecting the leukemogenic virus must be developed. Attempts will be made to detect the leukemogenic agent by means of a "helper" virus. Leukemogenic studies will be pursued in laboratory animals species for testing bovine leukemia research procedures.

Date Contract Initiated: June 30, 1965

Current Contract Level: $324,000

CORNELL UNIVERSITY (PH43-65-620)

Title: Leukemia Studies in the Cat

Contractor's Project Director: Dr. Charles G. Rickard

Project Officer (NCI): Dr. John P. Glynn

Objectives: The principal purposes of this project are to determine the etiology of spontaneous leukemia in cats, to determine means of transmission, and pathogenesis. Human leukemogenic material will also be studied for neoplastic effects in cats.

Major Findings: Electron microscopy has revealed typical C-type virus particles in five of seven spontaneous cases of generalized feline lymphosarcoma (lymphatic leukemia), but not in one case of localized lymphosarcoma involving the kidneys of a cat.

CORNELL UNIVERSITY (PH43-65-620)

Transmission has been accomplished from three spontaneous cases of feline lymphocytic leukemia by inoculation of newborn or very young kittens.

a) The Angell Memorial strain transmitted to three kittens to produce thymic and generalized lymphatic neoplasia, after latent periods of 9-12 months. Although cellular inocula were used, there is cytogenetic evidence that the induced tumors were not transplants of donor tissue.

b) The F-11 strain transmitted both by cellular and cell-free methods, producing lymphosarcoma after latent periods of 14-17 months. C-type virus present.

c) The F-161 strain transmitted through three serial cell-free passages by Moloney centrifugates, and has been filtered through a 0.8 micron Millipore filter. Induced lesions were a proliferation of reticulum and histiocytic cells in lymph nodes, spleen, and other organs. Incubation periods were 30-104 days, usually about 60 days. Numerous C-type virus particles and buds.

Lipomas or liposarcomas occurred in three kittens inoculated with the F-161 strain of virus. Attempts are in progress with tissue culture to demonstrate a feline sarcoma virus.

Typical C-type virus particles were found in one uninoculated control kitten, housed with three inoculated littermates. It is not known if this represented a spontaneous C-type virus infection, or horizontal transmission from inoculated contact kittens.

Tissue cultures have been established from feline leukemic tissues for periods up to 8 months and 46 passages. C-type virus infection of the cultured cells has persisted for as long as 61 days and 9 passages, but usually for 2-3 weeks. Viremia has been demonstrated in plasma pellets and in thrombocytes. An ultrastructural study of normal cat leukocytes and platelets has been done. Leukemic tissues have been supplied to several investigators.

Significance to Biomedical Research and the Program of the Institute: Cat leukemia is a fairly frequently observed disease of cats. The close association of cats with humans suggests that the possible common etiology of human and cat leukemia be carefully investigated. In addition, cat leukemia studies will provide one of the most useful and practical leukemia model systems for research.

Proposed Course: Additional serial transmissions will be attempted. Spontaneous C-type virus infections in non-leukemic cats will be sought. Total body X-irradiation of kittens will be done to attempt to activate leukemia viruses. Experiments on horizontal transmission in cats will be done. A specific-pathogen-free colony is being developed. Tissue culture lines will be investigated for susceptibility to the feline leukemia virus strains. Serological studies will be done, in part with other investigators, to demonstrate group specific antigens.

Date Contract Initiated: June 23, 1965

Current Contract Level: $153,800

RESEARCH FOUNDATION OF STATE UNIVERSITY OF NEW YORK (PH43-65-605)

Title: Comparative Studies on the Chemical and Physical Nature of the Known Leukemia Viruses and their Relationship to Particles Recovered from Other Sources

Contractor's Project Director: Dr. Charles L. Burger

Project Officer (NCI): Dr. John P. Glynn

Objectives: To determine the physical and chemical nature of virus-like particles recovered from leukemic human and animal material, and to determine the relationship of these particles to viral particles recovered from other sources.

Major Findings: The major effort during this period has been concerned with samples derived from lymphosarcomatous cats, supplied by Dr. Charles Rickard of State University of New York Veterinary College at Cornell University. These samples have been examined by electron microscopy and by density gradient centrifugation.

Samples were supplied to Dr. Fink at NCI for testing against viral antigens. No positive data were obtained. Either the virus in these samples is different or the techniques have destroyed the antigenicity. Testing will be performed by running Rauscher and/or Moloney virus in the same fashion. These samples will be tested on a blind basis by Dr. Fink.

The amino acid analyzer has been installed and tested. Tissues are being prepared for analysis. Both viral and non-viral-containing tissues from bovine, human, feline and other sources to be determined will be examined.

Techniques for the isolation of DNA from RNA in a single column analysis have been tested and are now used routinely. This will permit definitive base ratio determinations on samples recovered in gradients.

Fatty acid analyses of viral-containing and non-viral-containing tissues have continued. Long chain fatty acids of length greater than Carbon 22 were found in viral-containing tissues but not in non-viral ones. It appears now that a concentration factor is involved and more of the unusual fatty acids may be present in the viral tissues than in the non-viral-containing ones.

Other techniques including electrophoresis have been explored preliminarily for producing "clean" virus. Herpes simplex virus will be utilized as a testing system. Immunofluorescence studies have begun using human and murine virus.

L-asparaginase is being tested for its ability to inhibit or reduce tumor formation after the application of Moloney sarcoma virus in BALB/c mice.

Significance to Biomedical Research and the Program of the Institute: The major emphasis in virus separation studies involves human and animal materials under the Special Virus Leukemia Program. Developmental Research on separative procedures with known murine leukemia viruses has led to the establishment of procedures used for isolation, purification and concentration of candidate human and other animal agents. In addition to physical and chemical characterization studies, purified materials are also supplied to other investigators within SVLP. The activities under this contract represent an essential component of a broader collaborative undertaking.

Proposed Course: First priority will be given to the feline material obtained from Cornell University. Studies will be initiated on the separation of viral subunits. These fractions will be tested biologically.

A continuation of the studies above is planned. A tissue culture laboratory which remains to be established will be used in biological testing. Immunofluorescence which has already begun will continue.

Date Contract Initiated: April 21, 1965

Current Contract Level: $96,589

TAFT SANITARY ENGINEERING CENTER, CINCINNATI, OHIO (PHS-VCL-30)

Title: Studies on Thermal Inactivation of Viruses in Milk and Milk Products

Contractor's Project Director: Dr. R. B. Read

Project Officer (NCI): Mearl F. Stanton, M.D.

Objectives: To determine inactivation rate of selected tumor viruses in heat-treated milk and milk products. To define specific control measures which would prevent the passage of active viruses through such procedures as milk pasteurization.

Major Findings: Thermal inactivation studies have been continued with emphasis on the heat treatment of viruses requiring either *in ovo* or *in vitro* assay. A total of 2400 samples of Moloney and Rauscher murine leukemia viruses as well as Rous sarcoma virus were prepared using the slug-flow heat exchanger developed for this purpose. Heating temperatures of 55, 60, 65, and 71.7 C were used for holding times of 2, 4, 6, 10, 12, 15, 20, and 30 sec. 1 x 10^4 pfu of adenovirus 12, *Herpes simplex*, Simian virus 40, and reovirus, suspended in sterile milk, did not survive a 5-second exposure at 71.6°C, which is commercial pasteurization temperature.

Results to date with Rous sarcoma virus show no significant loss in titer by 5- to 30-min. exposure to 40, 45, or 50 C when the virus is suspended in sterile milk. At 55 C the virus titer was reduced 4 $\log_{10}$ after 25-min.

heating and no virus activity was seen after 5 min. at 60 C. With ice cream mix, there was no significant loss in virus titer at 40 and 45 C. At 50 C there was a one-log reduction in virus titer after 30-min. heating; whereas, at 55 C no virus activity was detected after 15-min. heating.

Identification of ratios of the flow rate of the fastest particle to average for the slug-flow heat exchanger has been completed for heat treating raw milk, sterile milk, chocolate milk, and chocolate drink for virus inactivation determinations with 30-sec. holding time or less. This ratio ranges from 1.034 to 1.076 depending on the flow conditions and vehicle. The closeness of this value to unity makes this instrument ideal for thermal inactivation studies.

Significance to Biomedical Research and the Program of the Institute: Surprisingly little detailed investigation has been done on the inactivation of tumor viruses. The present study attempts to fill this gap in our knowledge, particularly in regard to thermal and ionizing radiation inactivation. Inactivation curves using both oncogenic response in vivo and other characteristics of the viruses in vitro are being carried out on Simian Virus 40, adenovirus 12, leukemogenic RNA viruses of the Moloney and Rauscher types, reovirus 1, herpes simplex, murine sarcoma virus and Rous sarcoma virus. Of particular importance from a public health standpoint are the heat inactivation curves in milk and other dairy products. Of future importance, the data obtained will be valuable for studies on virus attenuation and vaccine development.

Proposed Course: This contract was completed on June 30, 1968.

Date Contract Initiated: FY-1966

Current Contract Level: $85,000

EINSTEIN MEDICAL COLLEGE, NEW YORK (PH43-65-612)

Title: Research on Immunological Factors in Susceptibility to Murine Leukemia Viruses

Contractor's Project Director: Dr. Frank Lilly

Project Officer (NCI): Dr. John Glynn

Objectives: To determine the role of genetic and immunological factors in the susceptibility of certain animals to candidate murine and human leukemia viruses.

Major Findings: Completed studies indicate that two separate genes determine absolute susceptibility to the early splenomegalic response induced by the F-S and by the F-B strains of FV, and that these genes (with alleles S^S and R^S for susceptibility and resistance to F-S virus and alleles S^B and R^B for F-B virus) segregate independently of each other and of the H-2 gene. The DBA/2 strain is homozygous for both the S^S and S^B alleles, and we are well into a breeding program designed to separate these two susceptibility alleles between two new strains: the DBA/2-R^S, which will be genotypically R^SR^S and S^BS^B, and the DBA/2-R^B, which will be S^SS^S and R^BR^B.

H-2 type has a marked influence, in mice genotypically S^B/-, on the threshold virus dose necessary for the induction of the splenomegalic response with F-B virus. Further, at any given level of virus dose, H-2 type has a pronounced influence on the prognosis for recovery of a mouse with F-B virus-induced splenomegaly.

An assay for substances bearing the FMR antigen activity has been standardized, using the release of Cr^{51} from labelled test cells by anti-FMR antiserum as its basis. The soluble FMR antigen present in the serum of FV-infected mice is not detectably associated with the virus particles also present in the serum. The virus-neutralizing antibodies present in hyperimmune FV antisera are separate from the cytotoxic anti-FMR antibodies. FMR antigenic activity appears to reside on at least two molecules of different sizes which are separable by chromatography on Sephadex G-150.

Significance to Biomedical Research and the Program of the Institute: One of the major problems in experimental transmission studies is the identification of susceptible animals or strains of animals. In no small measure the rapid progress with the leukemogenic agents in mice can be attributed to availability of inbred susceptible strains.

The present studies are concerned with the genetics of susceptibilities to leukemogenesis in mice and the possible application of these studies in attempts to transmit leukemia in other species.

Proposed Course: These studies will be pursued further during the coming year. The effect of retardation in the F_1 hybrids could reflect merely an aspect of heterosis, or "hybrid vigor". Experiments will be made to determine if the effect will be seen in F_1 hybrids of two susceptible $H\text{-}2^k$ strains of independent origin. It is also planned to make use of this seemingly sensitive F1-test system as a simple and relatively rapid assay of the comparative effect on susceptibility of new H-2 alleles and also in the attempt to solve the problem of the mechanism of action of the H-2 gene in its effect on GV susceptibility.

It is also intended to determine by studies in segregating generations if the difference in the rates of regression of MSV tumors in newborn BALB and C57BL mice might also be associated with H-2 type.

A breeding program is now under way to establish the following congenic mouse strains: (1) a DBA/2-R^B strain which will be susceptible to F-S but resistant to F-B virus, and (2) a DBA/2-R^S strain which should be susceptible to F-B but resistant to F-S virus. The availability of these strains will permit studies of the interactions of these genes with each other and with the H-2 locus. This breeding program is advancing rapidly, and it is estimated that about one more year will be required for its completion.

Date Contract Initiated: May 13, 1965

Current Contract Level: $76,135

NATIONAL COMMUNICABLE DISEASE CENTER, ATLANTA, GEORGIA (NCI-VCL-(68)-42)

Title: Etiologic Studies of Leukemia and Related Diseases Occurring in Unusual Epidemiologic or Genetic Situations

Contractor's Project Director: Clark W. Heath, Jr., M.D.

Project Officer (NCI): Dr. John B. Moloney

Major Findings: Activities during the year period have included: (1) continuation of leukemia case surveillance programs in 9 pilot areas in the United States (metropolitan areas of Atlanta, Houston, Kansas City, Minneapolis, Alameda County, California, states of Utah, Washington, Connecticut and Rhode Island) with initiation of an additional program for surveillance of childhood leukemia in Nassau County, New York; (2) investigation of leukemia cases occurring under unusual epidemiologic or genetic conditions. A total of 8 unusual situations were studied in which specimens of serum, buffy coat, marrow or other tissues were obtained for virologic studies. Investigations covered 3 leukemia case clusters, 2 instances of familial leukemia (one in association with mongolism), one instance of leukemia following blood transfusions, one instance of leukemia in a man and his dog and one community-wide survey in which specimens of serum were collected.

A total of 49 specimens were obtained for cell culture and electron microscopy at the Pfizer laboratories in Maywood, New Jersey. Autopsy material from one dog with lymphosarcoma was taken to the NCI for study. In the course of the community survey 685 sera were collected and stored at the NCDC Serum Bank.

Significance to Biomedical Research and the Program of the Institute: The EIS, CDC, has an excellent staff of medical epidemiologists stationed in Atlanta and throughout the United States who are able to conduct field investigations on short notice in either domestic or foreign locations. During the past 6 years, they have often cooperated in the collection of plasmapheresis specimens from community leukemia case-cluster situations, animal-human leukemia case associations, cases of familial leukemia, and leukemic twins. The proposed agreement with the CDC is designed to insure systematic collection and study of such specimens under the guidance of Dr. Clark W. Heath. It should also be noted that the CDC has ample facilities for storage and preservation of biologic specimens.

Proposed Course: During FY 1969, investigations of selected leukemia cases or cases of potentially related diseases will continue to be pursued, and appropriate biologic specimens will be collected.

Date Contract Initiated: July 1, 1967

Current Contract Level: $85,200

THE JACKSON LABORATORY, BAR HARBOR, MAINE (PH43-67-744)

Title: Murine Leukemia-Sarcoma Complex: Natural Occurrence of Leukemia Virus and the Sarcoma Genome in Mice

Contractor's Project Directors: Dr. Hans Meier and Dr. David D. Myers

Project Officers (NCI): Michael A. Chirigos, Ph.D.
Robert J. Huebner, M.D.

Objectives: (1) Detection, diagnosis, and propagation of spontaneous tumors of inbred strains of mice; (2) isolation of leukemia and sarcoma viruses or both from tumors and normal mouse tissues; (3) studies of the natural prevalence, incidence, and spread of leukemia viruses; (4) rescue of sarcoma virus genomes; (5) determination of the comparative sensitivities of embryonal cells from different inbred strains for leukemia virus isolation; (6) investigation of the effects of endogenous interferon production and release by Statolon on leukemia development (Statolon therapy); and (7) the murine viral spectrum of our mouse colonies.

Major Findings: Detection, diagnosis, and propagation of spontaneous tumors: Of 173 tumors biopsied and diagnosed histologically, 76 were mammary adenocarcinomas in various strains, 9 were mammary myoepitheliomas, 1 a mammary ductular squamous cell carcinoma, and 39 were lymphoid and lymphoreticular neoplasms. The remaining 48 tumors represent a variety of pathological types including fibro- and rhabdomyo-sarcomas.

Isolation of leukemia and sarcoma virus(es): Primary and transplanted tumors, organs of tumor-bearing mice, and tumor-derived tissue culture cell lines have been surveyed for MSV-CF-antigen(s). All but 5 tumors are CF-positive. Virus isolation from CF-positive tumors proceed both *in vitro*, and *in vivo*. Moloney concentrates have been inoculated into TC and newborns of isologous and at least 2 homologous strains. Bioassays of 3 virus isolates are underway. Virus isolations have been made *in vitro* from an LAF_1-rhabdomyosarcoma, and from RF/J retired breeders, C57BL/6J pregnant breeders, and from high leukemia strains AKR/J, C58/J, and SJL/J. Production of MSV(AKR/J)- MSV(C58/J)-and MSV(SJL/J)-pseudotype virus, and of virus pools are in progress.

Studies of the natural prevalence, incidence, and spread of leukemia viruses: Extensive natural history studies are being conducted in inbred strains, with and without tumors, among the mouse colonies, and the holding facility for retired breeders. These mice are being surveyed for MSV/CF-reactive materials. Analysis of results, consisting of CF-titers on several hundred mice from 12 strains, takes into account strain relationships by room, section, and pen, with high leukemia strains. Two cases of lymphoblastic leukemia in SWR/J male mice, exposed 49 and 90 days, to high leukemia strains RF/J, SJL/J, and C58/J were found. Virus excretion studies are being conducted among animals of this same environment.

Rescue of sarcoma virus genome(s): 5 MSV/CF-negative tumors including a fibro- and rhabdomyosarcoma are of considerable interest because of the possibility that they contain (a) defective sarcoma virus genome(s). Attempts at rescue in vivo have been initiated.

Statolon studies: AKR/J and SJL/J mice of leukemia age are treated with Statolon, hoping to prolong life and permit development of sarcomas containing defective sarcoma virus genomes. It is planned to treat 4 to 5 week old AKR/J mice using a dose of 15 mg/mouse every 10 days throughout their lives, hoping to prevent development or postpone the onset of leukemia.

Significance to Biomedical Research and the Program of the Institute: This contract, although effectively in operation for much less than a year, has already contributed significantly to the SVLP. The recovery of leukemia virus from low as well as from high leukemia strains has pointed up the necessity to re-evaluate and re-interpret previous data on the etiology of murine leukemia and cancer. Thus all of the viruses isolated from normal tissue and tumors from high and low incidence mouse strains at JAX and elsewhere have turned out (so far) to fall into the Gross (G+) or AKR serotype.

This contract provides an excellent opportunity for delineating modes of natural spread of the "C" type leukemia-sarcoma complex viruses and the clinical consequences of both horizontally and vertically acquired infections. It is hoped to better define the roles of naturally acquired viruses in the etiology of spontaneous solid tumors in genetically distinct strains of mice; and at the same time to determine the supplementary roles of genetically different susceptibilities of the JAX mice in the natural spread of viruses and in their clinical expression when they do cause infection.

Proposed Course: Continue, expand, and complete the numerous experiments in progress. Major emphasis will be placed on virus isolation, production of virus pools, virus bioassay, and rescue attempts of defective sarcoma virus genomes.

Date Contract Initiated: May 2, 1967

Current Contract Level: $82,000

FLOW LABORATORIES, INC., ROCKVILLE, MARYLAND (PH43-67-1396)

Title: Isolation and Characterization of Canine Lymphoma Viruses

Contractor's Project Director: Dr. Raymond V. Gilden

Project Officers (NCI): Michael A. Chirigos, Ph.D.
Robert J. Huebner, M.D.

Objectives: To isolate and characterize canine lymphoma viruses, and pursue basic studies of the murine leukemia-sarcoma complex as follows: (a) attempts at tissue culture isolation and assay of leukemia viruses from spontaneous canine lymphoma and other dog tumors; attempts to induce neoplasia in dogs using murine leukemia and sarcoma viruses; (b) immunological studies (fluorescent antibody, complement fixation) of the antigenic structure of "defective viral genomes "rescued" from non-virus shedding mixed cultures; preparation of quantities of serological reagents against envelope antigens and "internal" antigen(s) of leukemia viruses.

Major Findings: A transplantable beagle mastocytoma was obtained from Dr. C. Rickard of Cornell University. Transplant passage lines have been established in both newborn and weanling beagles. Tissue culture lines from a number of tumors have also been established. Cell-free transmission experiments are now in progress but are thus far negative.

Serological studies, complement-fixation (CF tests) employing a variety of canine sera and extracts of canine primary tumors (7 osteosarcomas or osteogenic sarcomas) and tissue cultures of these tumors, have been negative. Complement fixation tests using murine leukemia broad reacting sera and extracts of canine tumors and tissue cultures have also been negative.

A variety of attempts to isolate a "sarcoma" virus (focus forming agent) on beagle embryo cells either directly or in "genome rescue" experiments using extracts of a lymphoma culture as helper virus have also been negative. An RNA containing particle has been demonstrated by tritiated uridine uptake and sucrose gradient centrifugation in cultures of a German shepherd lymphoma. Other canine tumor and control cultures are thus far negative. Assays for the murine leukemia and sarcoma viruses have been established.

Significance to Biomedical Research and the Program of the Institute: Since the dog has a very close domestic relationship to the human, and since there have been some leukemic dogs who have been owned by children or persons who have also developed leukemia, it is important to isolate and characterize canine lymphoma viruses because of possible etiological relationships. If cell-free transmission of canine tumors can be effected, important information will have been gained in the search for a canine leukemic agent. There is also considerable need for devising biochemical and immunological methods for detecting "C" type RNA virus genetic information in tumor cells, since this is related to the problem of rescue of viral genomes. The proposed studies with the murine leukemia-sarcoma complex would undoubtedly provide basic information which may be applied to leukemia-sarcoma virus relationships in other animals, possibly including man. Basic immunological studies on relevant antigenic structures are also needed at present.

Proposed Course: Continuation of current efforts to demonstrate cell-free transmission of canine tumors, primarily the mastocytoma. Application of isotopic procedures to detection of overt and covert leukemia-sarcoma viruses or genomes in canine tumor cells. Continuation of efforts to detect specific complement-fixing antigens in canine tumors.

Date Contract Initiated: June 20, 1967

Current Contract Level: $149,700

UNIVERSITY OF MIAMI SCHOOL OF MEDICINE (PH43-67-1187)

Title: Vaccine Studies on Avian Leukosis and Related Problems

Contractor's Project Directors: Joerg A. Jensen, M.D.
M. Michael Sigel, M.D.

Project Officer (NCI): Dr. John P. Glynn

Objectives: To develop vaccination procedures against avian leukosis, including such specific approaches as the following: (1) immunochemical characterization of antibodies to avian leukosis virus under conditions of "natural" infection; (2) determination of the temporal synthesis of antibodies in the chicken in response to virus-containing antigens, and to leukosis and tumor cellular antigens; (3) immunochemical separation of various classes of antibodies to determine their relative efficacy against avian leukosis, Rous sarcoma, and influenza PRS viruses and tumor cells; (4) determination of the conditions of potential vaccine administration that will enhance the immune response, particularly those conditions favoring production of the most effective class of antibody molecule; (5) preparation of vaccines by various means to potentiate host response. In addition, the Contractor shall devote a limited effort, as time permits, to evaluating the feasibility of using adenovirus type 12-induced tumors in hamsters as a model for investigating immune tolerance in viral oncology.

Major Findings: The in vitro focus forming assay of RSV is now working well and titrations of RSV stock have been in close agreement with in vivo tumor-producing activity. The first neutralization test is in progress and will be correlated with in vivo results with the same serum. A crude stock of RSV-RAV 1 has been prepared in Spafas chick embryo fibroblasts and will be used to prepare a potential vaccine.

Neutralization tests in the chick wing web have shown serum from newly hatched conventional chicks to contain maternal, passively acquired antibody to RSV-RAV 1, whereas Spafas chicks have no antibody. The antibody has been identified by gel filtration and 2-mercaptoethanol-resistance as 7S IgG. The same class of antibody has been demonstrated in high concentrations in the yolk of eggs from conventional chickens. To study further the "natural" development of antibody in chickens, flocks have been maintained in different environments: (a) lab animal quarters, (b) an isolator, (c) outdoors - near lab., (d) a small commercial chicken farm and (e) a suburban yard. Selected pools of sera from these animals have been tested for neutralization of RSV-RAV 1. The following findings have emerged: (1) all animals lose maternal antibody at about 16 days; (2) antibody has reappeared after about 48 days in animals maintained in all environments except those on the chicken farm; and (3) in a given environment antibody reappeared only in some of the animals -- not in all. These observations are being further investigated for possible significance in regard to large scale immunization. New antibody was found to appear at 14-18 days after inoculation of RSV-RAV 1. At 25 days after virus inoculation, and in the presence of large Rous tumors, the animals contained relatively high levels of antibody, the great majority of which was determined to be IgG.

Using influenza virus as a tool, it has been determined that the chicken can respond to virus inoculation with IgM as well as IgG. These data will be extended for application toward producing IgM antibody in response to RSV and avian leukosis vaccines.

Preliminary studies have been done with chicken complement. Certain incomparabilities between chicken antibody and guinea pig complement have been found. Early results indicate the feasibility of utilizing chicken complement in cytotoxicity and complement-fixation tests.

Significance to Biomedical Research and the Program of the Institute: This contract seeks to develop important information directed specifically at two major objectives of the SVLP (1) using animal systems, determine the feasibility and effectiveness of vaccines for the prevention of virus-induced cancers, and (2) if some human leukemias are induced with animal leukemia viruses, determine whether this transmission chain can be interrupted by vaccinating or by some other means controlling the virus and its induced disease in animals. In view of the fact that avian species are highly susceptible to infection and disease induction by at least four different oncogenic viruses and that these animals (or their food products) are in close and prolonged contact with man, this study has important applicability.

Proposed Course: Continue above studies for another year at least.

Date Contract Initiated: June 23, 1967

Current Contract Level: $76,197

GERMFREE LIFE RESEARCH CENTER (PH43-65-95)

Title: Operation of a Collaborative Germfree Tumor Virus Laboratory

Contractor's Project Director: Dr. C. O. Prickett

Project Officer (NCI): Dr. W. Ray Bryan

Comment on change in objectives and title of contract. Due to the terminal illness and death of the former Project Director, Dr. James A. Reyniers, during the year the activities under this contract were reoriented in their entirety to serve immediate high priority needs of the SVLP. Although previously an important collaborator in SVLP activities, a substantial part of the effort of the contractor had involved research on germfree systems and methodology directed by Dr. Reyniers, and projected toward future needs of germfree life research.

Objectives: (1) To study neoplasms induced by chemical carcinogens in germfree quail (Coturnix), chickens, mice and rats, particularly with respect to the recovery of oncogenic viruses or the activation of latent viruses; and to supply such tumor-bearing hosts to other SVLP participants for oncogenic viral genome rescue attempts. (2) To study known oncogenic RNA viruses in their germfree natural hosts, particularly at low dose levels approximating naturally transmitted doses, in which the virus may be present only in a "hidden" state in the induced disease; and to suppl- germfree hosts with "noninfective" tumors to other SVLP participants for studies on recovery of virus from the "hidden" state. (3) To develop and characterize germfree and "reagent grade" specific pathogen-free (SPF) laboratory animals of strains or stocks required for the above and other SVLP program activities; and to supply such characterized breeder animals to other SVLP contractors for quantity production.

Major Findings: (1) Transplantable tumors of germfree quail initially induced with methylcholanthrene in benzene solution (MCA/Bz), and primary MCA/Bz-induced tumors in the same host, are being produced and supplied to other SVLP contractors and participating groups who are collaborating toward the common goal of detecting and rescuing "hidden" C-type RNA oncogenic viruses, if such be present. This collaborative project is of recent origin and no final results are as yet available. (2) Exploratory studies of the Schmidt-Ruppin strain of Rous sarcoma virus (RSV) in germfree quail showed a high percentage of regressing tumors, with evidence of considerable host reaction against the virus-induced lesions, thus indicating that this strain of RSV is not suitable for studies toward objective (2), given above. Similar studies on other potentially useful avian sarcoma virus strains are now in progress. (3) Developmental studies on the production of germfree chickens from eggs of leukosis virus-free hens of the Luginbuhl White Leghorn SPF Colony have recently been initiated. When germfree chickens are available, tumors will be induced with MCA/Bz and supplied to investigators as described above for quail.

Developmental research is also in progress to carry two present germfree lines of inbred mice (BALB/c and ALB/2) through additional cycles of caesarian section and aritificial feeding, in attempts to rid them through selection from vertically transmitted viral agents of the C-particle type. A colony of NIH mice (non-inbred) is also being built up to supply pregnant females for attempts to develop a germfree line from this stock, which appears to be free at least from infectious viruses of the C-particle type, and therefore of extraordinary value to many research activities of the SVLP.

Significance to Biomedical Research and the Program of the Institute: New developments in the problem of recovery of "hidden" oncogenic viruses involve a phenomenon first discovered in avian systems and then shown to apply also to murine systems. It is the existence in a defective or noninfectious state of certain C-type sarcoma viruses, and their dependence on coinfection with a "helper" virus in a dual infection for their replication in an infectious form. The "helper" viruses are C-type leukemogenic viruses and certain of their nonleukemogenic "orphan" relatives.

The study of interactions among such viruses for acquiring basic information that may lead to recovery of similar human viruses from the "hidden" state requires: (1) that the experiments be performed in animal hosts that do not carry their own flora of extraneous potentially interactive agents, and (2) that the experimental animals be protected against intercurrent infection with such agents through the course of the investigations. The collaborative contributions of this contractor provide these desiderata in a broad joint study involving also various other specialized groups of investigators who are participating in the SVLP.

Proposed Course: The operation of this collaborative germfree tumor virus laboratory will be continued, with the flexibility of adjusting as rapidly as possible to new developments and the pursuance of prime SVLP objectives.

Date Contract Initiated: April 16, 1965

Current Contract Level: $267,275*

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* Yearly rate, reduced by unexpended balance of $55,000 of previous contract year.

RUTGERS, THE STATE UNIVERSITY (PH43-67-1166)

Title: Etiology of Marek's Disease (Avian Neurolymphomatosis)

Contractor's Project Director: Dr. Vincent Groupé

Project Officer (NCI): Dr. W. Ray Bryan

Objectives:

(1) To study further the conditions under which the disease can be transmitted in the laboratory.

(2) To isolate and characterize the etiological agent involved.

Major Findings:

The following progress has been made toward the end objectives:

(1) Establishment of a tissue culture laboratory exlusively for work with Marek's disease materials.

(2) Initiation of field studies at nearby poultry farms for identifying infected flocks as sources of field cases of Marek's disease for laboratory investigations.

(3) The initiation of transmission studies using the GA (laboratory) strain of Marek's disease, both in tissue culture and in chickens.

The etiological agent apparently is either highly cell associated or unusually unstable to the usual procedures of isolating virus in a cell-free state. Cell-free transmission of Marek's disease has not yet succeeded in any laboratory. Studies on the factors involved, directed toward isolation of the agent, are being pursued.

Significance to Biomedical Research and the Program of the Institute:

Marek's disease is considered to be a potentially valuable new model for uncovering oncogenic viruses which may be present in a "hidden" state. Since the disease is known to be transmitted in nature, a specific oncogenic agent is known to be associated with it. The development of technology which will lead to its isolation may lead to knowledge of a new state of, or conditions for, virus "hiding", and to methodology which can also disclose "hidden" viruses of certain types in neoplasia of human and other animal species.

Proposed Course:

This program was initiated as a high priority objective at the beginning of this fiscal year. It will be continued at essentially the same level of operation.

RUTGERS, THE STATE UNIVERSITY (PH 43-67-1166)

Date Contract Initiated: 6/9/67

Current Contract Level: $97,816

SOLID TUMOR-VIRUS PROGRAM SEGMENT

Dr. Robert J. Huebner, LVD, NIAID, Chairman
Dr. James T. Duff, VCB, Etiology Area, Vice Chairman

ATOMIC ENERGY COMMISSION, OAK RIDGE NATIONAL LABORATORY (FS-7)

Title: Centrifuge Development and Cellular Anatomy Program

Contractor's Project Director: Dr. Norman G. Anderson

Project Officer (NCI): Dr. Charles W. Boone

Objectives: (1) To conceive, plan, and develop zonal ultracentrifuges and related accessory equipment for the separation of subcellular components, viruses, and nucleic acids in pure fractions from biological materials; (2) to employ the engineering capabilities of the staff of the Oak Ridge National Laboratory in the conception, design, fabrication and testing of instruments needed for specific biomedical research needs of NCI; and (3) to evaluate the products of (1) and (2) with selected biological systems.

Major Findings:

Development of A Series Rotors: Rapid methods for isolating nuclei are of interest for the isolation of repressor RNA and DNA in a relatively undegraded form. The development of reorienting gradient rotors suitable for this purpose has been explored with the A-XVI rotor. An even more rapid separation is achieved in the A-XVII which is loaded and unloaded dynamically with great rapidity using scoops in place of seals. With this rotor the problem of very rapid large-scale isolation of nuclei appears to be reaching a solution. A simplified version of the A-XII rotor (designated A-SVIII) has been developed which does not require seals, yet has all the advantages of the transparent A-XII rotor.

Development of B Series Rotors: Drawings of the B-XIV and B-XV rotors have been released to industry and are now being manufactured by four different centrifuge firms. The B-XXIII rotor, designed for both center and edge unloading, has been used successfully for the separation of plasma membranes, mitochondria, and lysosomal activities. Modifications in the design have been incorporated in the B-XXIX rotor.

Development of K Series Centrifuges: The major effort during this year has been in the development of the large capacity

K series centrifuges for vaccine production. In practice, the K-II centrifuge separates grams of virus from kilograms of impurities. The armor of the K-IIB proved inadequate during destructive testing and was therefore redesigned completely with tubular knavel armor. This redesign alters quite markedly many of the operating procedures, but will allow much heavier and higher speed rotors run in the future. In collaboration with a pharmaceutical firm, over 2000 hours of operating experience was gained and over one-half million doses of influenza vaccine prepared. Additional rotor modifications (K-IV and K-V) were designed and tested which allow rate zonal experiments to be run in this centrifuge. A coaxial double-pass continuous-flow-with-banding rotor (K-VI) was constructed and evaluated with test particles. The purpose of the rotor is to give even higher purity in large-scale virus vaccine preparations. It should be noted that the K series centrifuges do not exhaust either the requirements or the technological possibilities of high speed continuous flow centrifugation.

A new technique termed "centriphoresis" has been developed in which electrophoretic separations are carried out in a centrifugally stabilized gradient. This offers a number of advantages in that larger gradients may be used and higher currents since the configuration allows for more efficient coding.

Zonal centrifuges produce large numbers of fractions for analysis. The development of automated analytical systems is therefore of prime importance. Essential ingredients of such systems are devices for accurate measurement and transfer of small fluid volumes. The first new accurate measuring and transfer system developed in the last 20 years has been developed for this purpose, has an accuracy of ±0.5% and is not affected by changes in solution density, viscosity, surface tension, or the presence of air bubbles.

Work on the isolation and characterization of the soluble and membrane bound T-antigens of adenovirus 31 hamster tumors is continuing. For this preparative high resolution electrophoresis is required and is being developed. The membrane bound antigen has been shown to be released in a soluble form by papain digestion. Definite immunization against tumor challenge was obtained in young hamsters by injection of tumor membrane fractions.

Significance to Biomedical Research and the Program of the Institute: The development of specialized centrifuges by the contractor for the quantitative separation of cellular components and viruses from biological materials represents a very significant technical advance for virology. Adenovirus type 31 tumor antigens and transplantation antigens are being used as model systems for adapting these instruments to solving problems encountered in this area of research.

Proposed Course: The major efforts during the next year include: (1) Intensive efforts to develop rapid *in vitro* assays for transplantation antigens from tumors; (2) continued development of centrifugal systems for cell fractionation and vaccine production; (3) development of intermediate and large-scale cell culture systems, and (4) continued studies on rotor systems for separating high-molecular-weight DNA on a sedimentation rate basis.

Date Contract Initiated: July 1, 1962

Current Contract Level: $850,000

ELI LILLY AND COMPANY (PH43-65-570)

Title: Deoxyribose Nucleic Acid (DNA) Virus Production

Contractor's Project Director: Dr. J. Paul Burnett

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: Using a human cell line of non-malignant origin the contractor produces radioactively labeled DNA from selected DNA viruses, purifies these, and supplies NCI investigators with the product.

Major Findings: The base sequence homology was determined for various simian adenoviruses. It was found that the oncogenic viruses SA-7 and SV-20 were very closely related to each other and much less related to the non-oncogenic types SV-32 and SV-39. The non-oncogenic types, however, were quite closely related to each other.

In the examination of the DNA of adenoviruses SV-32, SV-38 and SV-39 it was found that the guanine and cytosive content of these viruses (approximately 55%) was considerably lower that that of SA-7 (50%) and SV-20 (66%). At this laboratory SV-32 and SV-39 are non-oncogenic in newborn hamsters whereas SV-38, SA-7, and SV-20 are capable of inducing tumors.

Several clones of simian adenovirus type SA-7 have been isolated which differ in oncogenicity. Many of these clones were isolated from either the original wild-type strain or the wild-type that had been exposed to mutagenic agents and have a marker such as plaque type or temperature sensitivity. Nine mutants of SA-7 were found which do not induce tumors in newborn hamsters at dose levels comparable to those inducing tumors in 100% of the animals injected with wild-type SA-7. In addition, a few strains were isolated which induced tumors somewhat more rapidly than the wild-type.

In the last year the contractor produced 271.2 mg of purified adenovirus, 8.3 mg of which was tritium labeled; 100 mg of purified SV40 virus, 44 mg of which was tritium labeled; and 20 ml of high titer crude adenovirus. This was distributed to various NIH scientists in 19 separate shipments.

Proposed Course: The contract was terminated on January 31, 1968, since the contractor did not submit a renewal request.

Date Contract Initiated: June 14, 1965

Current Contract Level: $145,150

FLOW LABORATORIES, INC. (PH43-65-634)

Title: Preparation, Testing and Application of Specialized Adenovirus Serological Reagents to Human Cancer Screening Programs.

Contractor's Project Director: Dr. Raymond V. Gilden

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: Predicated on the discovery of the oncogenic properties of human adenoviruses in hamsters and in view of the extreme prevalence of adenovirus infections in man, to develop and provide specialized adenovirus serological reagents, as follows: (1) Viral or V subunit antigens and antisera. The V antigens are associated with adenovirus infections; (2) T-antigens -- those associated with virus-induced tumors and cell transformation -- free of virion; and (3) adenovirus-associated virus (AAV) reagents. The AAV's are common contaminants of adenovirus stocks.

Major Findings: The original goals of the contract - the development of methodology for preparation of adenovirus T-antigens for the oncogenic adenoviruses free of virion (hexon) antigens and the adeno-associated viruses (AAV's) - have for the most part been achieved and current emphasis is on production of reagents. Type specific antisera is now available and has been supplied to other NCI contractors to all four known AAV types. Hexon antigens free of detectable T and penton antigens have been prepared for adenovirus types 3, 7, 9, 11, 12 and 14. T-antigens are being produced for representatives of the three known adenovirus T-antigen groups. Adenovirus types 2, 7 and 12 T-antigens have been prepared in KB cells. The type 12 antigen was highly purified by physical means and reacted to high titer specifically with tumor antiserum. The type 2 and 7 T-antigen preparations were made in the presence of hydroxyurea and contained high T titers and minimal virion antigen titers. These preparations were submitted to the Project Officer for preliminary testing of human sera. Procedures for the separation of hexon and penton antigens of adenovirus types 2 and 7 have been established.

Significance to Biomedical Research and the Program of the Institute: These serological reagents are being used by the contractor and other scientists collaborating with the Solid Tumor-Virus Segment in sero-epidemiological studies of human cancer to help clarify what role, if any, these viral agents play in human neoplastic disease.

Proposed Course: The contractor will continue the purification of adenovirus 2 and 7 T-antigens, purification and specific antiserum production for 2, 7, 12 hexon and penton antigens, attempt to produce T-antigens and antisera for the non-oncogenic adenoviruses, and initiate production of T-antigens without V antigens for the oncogenic adenoviruses of infra-human origin.

Date Contract Initiated: April 27, 1966

Current Contract Level: $165,000

GEORGE WASHINGTON UNIVERSITY (PH43-64-515)

Title: (a) Adenovirus Purification and Concentration
(b) Procurement of Tumor Tissues and Sera from Cancer Patients

Contractor's Project Director: Dr. Ariel C. Hollinshead

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To separate, define and produce the specific T or tumor complement fixing (CF) antigens of the various oncogenic and non-oncogenic adenoviruses. The purified antigens finally produced will be designed for use in sero-epidemiological studies of adenoviruses as possible causes of human cancer.

Major Findings: Small amounts of group A adenovirus T-antigen, tumor antigen, and group B adenovirus T-antigen have been prepared for use in complement-fixation assays in the human cancer serological screening program. In addition, group C T-antigens, using adenovirus type transformed cells are being separated and studied. Additional antigens, which cannot be measured by complement-fixation and which are present in certain fractions after double passage by Sephadex gel filtration, are being investigated.

Significance to Biomedical Research and the Program of the Institute: Adenovirus T-antigens isolated and separated by this contractor have been submitted to Dr. Huebner's laboratory for use in a human cancer screening program.

Proposed Course: To continue studies on the preparation and separation of adenovirus group C T-antigens and to conduct investigations on the separation of tumor-specific, virus-induced transplantation antigens. The contract will terminate on June 30, 1968.

Date Contract Initiated: January 20, 1964

Current Contract Level: $180,230

MERCK INSTITUTE FOR THERAPEUTIC RESEARCH (PH43-64-55)

Title: Study of Viruses in Human and Animal Neoplasia

Contractor's Project Director: Dr. Maurice Hilleman

Project Officer (NCI): Dr. James T. Duff

Objectives: (1) To elucidate mechanisms of viral carcinogenesis; (2) to search for viral agents in human neoplastic disease; (3) to develop methods for prophylaxis of viral neoplastic disease.

Major Findings: Studies are in progress on the induction of virus from animal tumor cells of known viral origin and from human tumor cells by exposure of such cells to inactivated Sendai virus in the presence of appropriate "indicator cells" such as green monkey kidney and human embryonic kidney cells.

The testing of ordinary viruses and Mycoplasma for oncogenic potential in normal and thymectomized newborn hamsters has continued. None of the agents in the experiments still under observation have been found to be oncogenic. No additional agents will be studied.

Preliminary studies on the susceptibility of inbred hamsters to virus-induced tumor formation suggest that the MHA strain of hamster is less susceptible to SV40 oncogeneses than random bred hamsters. Another inbred line of hamsters, the LSH strain, is also being evaluated.

Complexed homopolymers (interferon inducers) are being tested for prophylactic and therapeutic effect in RNA and DNA virus-induced neoplasia of laboratory animals (Friend leukemia in BALB/c mice, SV40 and adenovirus type 12 tumorigenesis in hamsters). Results are not as yet available pertaining to prevention of adenovirus type 12 and SV40 virus tumors. With Friend leukemia virus, a group of mice receiving 10 doses of interferon inducer showed partial protection (60% mortality) in contrast to controls (85% mortality).

Previous reports indicated that adenovirus 7 tumor cells inactivated by formalin fixation, freeze-thawing or by French pressure cell disruption appeared capable of immunizing hamsters in the adult-cell challenge assay system. A second study was carried out in order to confirm this, and the results continue to indicate a protective effect.

Significance to Biomedical Research and the Program of the Institute: The studies of neoplasia carried out by this laboratory are aimed at the control of cancer in man by immunization and/or therapeutic means.

Proposed Course: Studies which are currently being pursued and which will be continued in the following period are: (1) Oncogenicity of viruses and Mycoplasma in newborn hamsters and thymectomized hamsters; (2) attempts to "induce" virus from animal and human tumor cells by the heterokaryon technique; (3) prophylaxis and treatment of experimental virus-induced neoplasia with polynucleotide interferon inducers; (4) assay of candidate inactivated or disrupted SV40, adenovirus, or chemically-induced tumor cell vaccines for protective efficacy in hamsters; and (5) search for a rapid specific *in vitro* test for assay of experimental tumor cell vaccines.

Date Contract Initiated: October 1, 1963

Current Contract Level: $305,574

SALK INSTITUTE (PH43-67-1147)

Title: Isolation of Temperature-Sensitive Mutants of Polyoma and SV40 Viruses

Contractor's Project Director: Dr. Renato Dulbecco

Project Officer (NCI): Dr. Charles W. Boone

Objectives: Isolation of Temperature-Sensitive Mutants of Polyoma Viruses

Major Findings: The different stages in the isolation of temperature-sensitive mutants for the polyoma virus are as follows: (1) Screening of mutagenized stocks at 33°C and 38°C for mutants; (2) plaque purification of suspected mutants, involving four serial single plaque isolations; (3) growth of purified mutants to high titer stocks; and (4) final testing of high titer stocks for leakage at high temperature and for the presence of wild-type revertants. Out of 50 mutants isolated so far, 15 have reached step 3 and 3 have reached step 4.

Significance to Biomedical Research and the Program of the Institute: Temperature-sensitive mutants of polyoma virus and SV40 virus can be used to provide unequivocal identification of the proteins induced by these viruses in the infected cell which are required to accomplish malignant transformation. This information should provide leads for elucidating possible molecular mechanisms of viral oncogenesis in humans.

Proposed Course: The remaining mutants are being plaque purified and will be grown to high titer stocks. After these stocks have been tested for leakage at high temperature, and for the presence of wild-type revertants, they will be made available to other scientists. Similar studies will be initiated with the SV40 virus.

Date Contract Initiated: June 5, 1967

Current Contract Level: $146,612

ST. LOUIS UNIVERSITY (PH43-67-692)

Title: Search for Viral-Specific Genetic Material in Human Cancers

Contractor's Project Director: Dr. Maurice Green

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: Dr. Green and his associates on this contract have continued to make significant progress towards their main objective, which is to detect viral genetic material in tumor cells. The hamster tumor and transformed rat cell models induced by the 12 oncogenic human adenoviruses (A, B and C group) have been used to demonstrate virus-specific mRNA transcribed in the tumor cells by the integrated non-infectious DNA's of these viruses.

Major Findings: The fact that 13 of the 31 human adenoviruses can transform cells with the incorporation or transcription of selected regions of the viral genome suggest the possibility that portions of adenovirus genomes may commonly be incorporated into human cells, perhaps producing certain malignancies and other disease. Since the formation of viral-specific mRNA is a common feature of transformation by human adenoviruses, a means is available for testing DNA viruses as causative agents of human cancer. It is feasible to examine human cancer tissue for the presence of viral mRNA by DNA-RNA hybrid formation or by hybridization-inhibition measurements.

Viral DNA's from the 31 human adenoviruses, SV40 virus, SV15 virus, bacteriophage P22, T7 and T2 have been prepared. These have been immobilized on membranes and checked for the presence of trace amounts of cell DNA. Membrane immobilized adenovirus DNA's provide good reagents for the detection of virus-specific RNA.

Adenovirus types 7, 16, 12, 2 and 5 tumor and transformed cell virus-specific RNA's labeled with ^{3}H-uridine have been prepared in yields of from 6 to 70 million cpm per 10^9 cells. These are being stockpiled for hybridization-inhibition analysis of human cancer RNA.

Seventy-two attempts have been made to label human tumor tissue directly with ^{3}H-uridine. In most cases less than 10^6 cpm were incorporated. Collagenase dispersal of tumor tissue appears to be useful since a several fold increase in ^{3}H-uridine incorporation has been observed in collagenase treated human tumors. Direct hybridization of labeled RNA from 21 human tumor, cancer, and leukemia leukocyte cultures with adenovirus, SV40, and SV15 DNA's have given several positive results; however, these must be further tested before their meaning is clear. One to 30 mg of RNA has been isolated from 204 human tumors; these represent 50-100% yields. Conditions for the hybridization-inhibition analysis of human cancer RNA have been established in model systems and provide very sensitive means for testing human cancers for virus-specific mRNA.

Significance to Biomedical Research and the Program of the Institute: This project represents the first direct opportunity for testing the association of DNA viruses with human tumors. These studies on the role of viral nucleic acid in cancer induction and diagnosis represent the most sensitive method available for determining whether a tumor is caused by a virus.

Proposed Course: In future studies on the hybridization analysis of human cancer RNA, this laboratory will systematically examine the incorporation of RNA precursors into human cancer tissue; will attempt to prepare at least two million counts per minute of RNA from each of 100 to 1,000 representative human cancer cell or tissue preparations for hybridization reactions with viral DNA's and RNA's; and will carry out competition hybridization analysis between human cancer RNA and labeled viral mRNA prepared from virus transformed and infected cells. In addition, efforts will be made to characterize the nucleic acid of the herpes-like virus isolated from Burkitt tumors.

Date Contract Initiated: March 20, 1967

Current Contract Level: $199,999

WISTAR INSTITUTE OF ANATOMY AND BIOLOGY (PH43-62-157)

Title: Studies of Diploid Cell Strains

Contractor's Project Director: Dr. Hilary Koprowski

Project Officer (NCI): Dr. Charles W. Boone

Objectives: (1) To determine the mechanism of SV40 virus transformation of human cells and the induction of neoantigens; (2) to determine the relationship of karyotypic instability to virus transformation of human cells; and (3) to quantitate the growth characteristics of normal and virus-transformed human cells by time-lapse photography.

Major Findings: Through the operation of this contract over the last five years, the WI-38 human diploid cell strain has been distributed to over 500 laboratories throughout the world and has become the most thoroughly characterized cultured diploid cell known. In the past year, 14 publications resulted from work done in the various subprojects.

In January 1968 the two projects pertaining to (1) the storage and distribution of WI-38 cells, and (2) the biochemical characterization of human diploid cells were terminated, and the contract was moved from the Resources and Logistics Segment, SVLP, to the Solid Tumor-Virus Segment.

Significance to Biomedical Research and the Program of the Institute: These studies should provide relevant information on the interrelationships of various antigens associated with DNA oncogenic viruses and their affected cells. This information should determine which antigen(s) or other properties of these viruses can be exploited to determine their role, if any, in the induction of human cancers.

Proposed Course: During the current contract year studies will be conducted on SV40 transformed human cells and tumor immunology, karyological instability of virus transformed cells, and the growth dynamics of human diploid cells.

Date Contract Initiated: February 2, 1962

Current Contract Level: $137,675

BIOHAZARDS & CONTAINMENT PROGRAM SEGMENT

Dr. Alfred Hellman, OASDVO, Etiology Area, Chairman
Mr. William E. Barkley, OASDVO, Etiology Area, Vice Chairman

UNIVERSITY OF MINNESOTA (PH43-65-999)

Title: Development of an "Open Isolation" System for the Care of Low Resistance Patients

Contractor's Project Director: Dr. George S. Michaelson

Project Officer (NCI): Mr. William E. Barkley

Objectives: To develop a "bioclean" area which would permit the domiciling of low-resistance patients. This facility will be of a conventional configuration, providing the patient with similar degrees of safety as attainable in "Life Islands", yet overcoming the psychological and physical limitation of the latter.

Major Findings: Recovered microbial contaminants from the "healthy patient" experiments were characterized and found to be types commonly associated with human skin and respiratory systems. The "healthy patient" was apparently the source of the air-borne contamination. The type and distribution of recovered microorganisms from nurses gloves was not consistent with the distribution of microorganisms recovered by air sampling. Interpretations of this observation and all other data from these experiments are still under study. The significance of glove contamination as a major factor in contamination transfer was confirmed. Gloves were sampled after operations involving no planned sepsis and contact with body tissues normally harboring indigenous flora. The majority of the gloves sampled were found to be contaminated. Characterization of the microbial contaminants suggests that man is the primary source. The source and degree of contribution from either patient or surgical team could not be fully elucidated.

Significance to Biomedical Research and the Program of the Institute: This project, which expands the "Life Island"^{R} concept to open isolation room systems, is closely coordinated with the NCI Medicine Branch staff who are responsible for the NCI "Life Island"^{R} projects. Results of this project continue to be promising. Design criteria and operational guidelines are being incorporated into intensive care isolation areas at NIH for treatment of leukemia patients. During the year, free consultation concerning the design of such a facility and the problems of adequate, valid microbiological monitoring in the bioclean environment has been provided to several major medical research facilities in the country.

Proposed Course: The initial objectives of this contract have been completed The final report will be prepared during the next triannual period. Patient evaluation, utilizing concepts and practices derived from this study, will be performed by the NCI Clinical Center. The study has identified many additional problem areas related to patient care which were not defined within the scope of this contract. Plans to pursue some of these newly defined objectives are underway. Two critical areas which we plan to study in the future are the problems associated with direct patient contact in laminar air flow environments and the effects of air velocity on unidirectional air systems.

Date Contract Initiated: June 22, 1965

Current Annual Level: $122,800

OHIO STATE UNIVERSITY (PH43-65-1001)

Title: Aerosol Transmission of Oncogenic Viruses

Contractor's Project Director: Dr. Richard A. Griesemer

Project Officer (NCI): Dr. Alfred Hellman

Objectives: The objective of this project is to elucidate the hazards to man and animals of aerosol exposure to oncogenic viral agents. Containment methods are well established; if used, they will prevent both exposure of man and cross contamination of experiments. Unfortunately, not enough is known about the conditions under which aerosol transmission occurs to know what degree of containment is necessary. Since containment equipment and methods are expensive and not available in all laboratories, indiscriminate containment is unnecessarily costly and time consuming. The contractor is continuing to evaluate selective oncogenic viral agents for their infectivity following airborn transmission and determing the fate of the virus after aerosol inhalation with emphasis on the routes and amount of viral excretion.

Major Findings: Both aerosol transmission and horizontal transmission have been demonstrated with viral titers approximating a minimal parenteral dose. Following Yaba virus infection, with 1 x 10^5 FFU/ml, monkeys developed asymptomatic pulmonary neoplasms. Yaba virus was re-isolated from all pulmonary tumors. The biological activity of Yaba tumors resulting from aerosol exposure differ significantly from those produced by subcutaneous, intravenous, intrapleural, or intrapulmonic injections. Pulmonary tumors developed in four weeks and persisted for at least 12 weeks after aerosol exposure in contrast with those produced by subcutaneous inoculation of comparable doses of virus which developed within 5 to 10 days and disappear by 4 to 5 weeks after infection. Pulmonary tumors produced by a viral aerosol did not metastasize while multiple visceral and subcutaneous tumors developed in monkeys infected intrapleurally or intrapulmonically. Continual reoccurrence of subcutaneous tumors at various sites occurred after aerosol exposure over a 3 to 4 month interval. Hamsters have demonstrated susceptibility to adenovirus 12 aerosol infection; this has been confirmed by the presence of T antigen.

Significance to Biomedical Research and the Program of the Institute: This project is essential for the development of biohazard design criteria. The demonstration that the Rauscher leukemia virus can be transmitted by aerosol: (1) suggests the need for isolating one experiment more completely from another, (2) infers that leukemogenic viruses may be hazardous to man and that they do infect laboratory animals via the aerosol route, thereby, indicating a hazard for laboratory animals and possibly the confounding of experimental results. Yaba virus does induce neoplasms via the aerosol route in subhuman primates (crossing species barriers), thus, direct implications for causation of human disease from this virus is apparent, and rigid containment of this virus becomes mandatory.

Proposed Course: To further evaluate the hazards associated with aerosol transmission of oncogenic viruses, the fate of a virus following aerosol inhalation will be determined with the Adeno 12 hamster as the model system. The susceptibility of animals with minor respiratory impairments to subsequent viral infection by the aerosol route will be investigated. This is extremely important since man and experimental animals nearly always have subclinical lesions in their respiratory tracts. It is conceivable that pre-existing lesions may serve as a foci for oncogenic virus infection as is the case with the lung mite and exposure to anthrax. Greater absorption and concentration may also occur in some histiocytic lesions.

Date Contract Initiated: June 22, 1965

Current Annual Level: $96,400

THE DOW CHEMICAL COMPANY (PH43-65-1045)

Title: Research and Development of Biohazards Containment Facilities

Contractor's Project Director: Dr. Guy C. Mattson

Project Officer (NCI): Mr. William E. Barkley

Objectives: The development of new concepts in the control and containment of hazardous agents to assure personnel protection and experimental integrity by providing facilities, equipment and procedures required to assure prudent and sensible containment operations is the primary objective. The contract objectives, therefore, have encompassed evaluation of potential hazards; study of the state-of-the-art of control and containment; development of criteria for, and assistance in the design and construction surveillance of, new facilities; and development of protocols for personnel training and rational operation of such facilities.

Major Findings: A major effort of this contract period was the completion of the engineering design and preparation of specifications for the prototype Virus and Tissue Processing Laboratory. The completed engineering documents have been sent to prospective bidders. Engineering design and analysis is continuing on the Environmental Control and Biohazard Laboratory.

These prototype units will occupy a portion of the open bay area in Building 41. Fabrication and evaluation of prototype containment equipment is progressing. The prototype Mobile Laboratory designed as a total containment microbiology research laboratory is being field tested at the National Communicable Disease Center in support of an ongoing scientific program. A modified laminar flow hood is being subjected to various microbial tracer and engineering tests to determine its capacity for controlling contamination transfer. Dow contract personnel are developing test protocols which will be used to evaluate the biological integrity of the EVIF. Dow is continuing to provide day-to-day evaluation of the EVIF construction, and consultation on other containment projects related to the SVLP.

Significance to Biomedical Research and the Program of the Institute: This research and development contract is an integral part of the National Cancer Institute's Special Virus Leukemia Program. The continued effectiveness of the research effort of NCI is dependent upon the construction of a new virus research facility which incorporates advances in biohazards containment techniques. The contractor is responsible for a continuing effort in assessing the needs of the National Cancer Institute for new containment techniques and facilities, consistent with the changing scope of current research. The contractor is also compiling and updating a report on the state-of-the-art of containment, designing, fabrication, and installing prototypes of new concepts for high hazard laboratories and animal rooms.

Proposed Course: The contractor will continue to provide on-site evaluation of the construction progress of the EVIF and consultation related to containment problems of other SVLP projects. Prototype facilities will be evaluated and stressed using microbial tracer techniques and engineering tests. Prototype equipment will also be analysed for containment efficacy. A biohazard information retrieval system will be studied on a trial basis to determine the need of a Central Biohazard Information Center to serve the research community. It is still anticipated that the contractor will provide on-site technical competence for performing defined biohazard activities. This necessary competence is not currently available at NCI.

Date Contract Initiated: June 25, 1965

Current Annual Level: $297,069

PROGRAM MANAGEMENT

Dr. Frank J. Rauscher, OASDVO, Chairman
Dr. Robert Reisinger, OASDVO, Vice Chairman

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-68-705, formerly PH43-62-200)

Title: Development and Production of Reagents for the Identification of Tumors Induced by Viruses.

Contractor's Project Director: Johng S. Rhim, M.D.

Project Officer (NCI): Robert J. Huebner, M.D.

Objectives: In support of the Viral Carcinogenesis Branch's search for naturally occurring murine leukemia viruses (NIAID program of Drs. Rowe and Hartley; Jackson Laboratory Contract PH43-67-744; NIH trailer laboratory) to: (1) produce, assay and identify all leukemia and sarcoma virus strains isolated; (2) evaluate and improve standard leukemia virus assay procedures; (3) develop *in vitro* methods for detection of sarcoma and other solid tumor inducing genomes occurring as variants of murine leukemias; and (4) test leukemia virus inhibiting substances (i.e., Statolon made by Eli Lilly Company) *in vitro*.

Methods Employed: Contractor has applied and adapted the COMuL test, a tissue culture test recently developed by Hartley *et al* in the Laboratory of Viral Diseases, NIAID, to demonstrate leukemia viruses in the tissues of healthy as well as leukemic mice.

Major Findings: In less than a year, Dr. Rhim and his associates have successfully adapted the COMuL and focus-forming tests to large scale survey systems. The Project Director has found embryo cells from various strains of rats useful in the COMuL test, furnishing for some leukemia viruses more sensitive and susceptible systems than the mouse cells usually used. Highly sensitive procedures for detection of naturally occurring murine leukemia and sarcoma viruses are necessary requisites to any study of the natural history of these agents. Searches for similar viruses in the leukemias and solid tumors of man and his domestic animals will be based on observations made on the natural occurrence of the mouse viruses.

Dr. Rhim has also apparently succeeded in transforming rat and hamster cells *in vitro* with Rauscher leukemia virus and several newly isolated Gross (G+) viruses. These cells transplant in hamsters and rats and contain infectious virus.

The contractor's preliminary results show that Statolon inhibits replication of the Moloney and Rauscher leukemia viruses, and the Moloney sarcoma virus in mouse embryo tissue cultures.

Significance to Biomedical Research and the Program of the Institute: This contract serves as a basic resource for the Viral Carcinogenesis Branch's program on C type murine leukemia and sarcoma viruses. Hundreds of new leukemia and sarcoma virus isolates are being assayed and grown out in sufficiently large amounts for characterization and identification. As such, this contract provides the basic diagnostic services indispensable to the Branch's efforts to determine the role murine leukemia viruses play in the etiology of spontaneous and chemically induced tumors of the mouse.

Proposed Course: This contract will continue in the same logistic and developmental research roles as before except that it will also now support the Branch's efforts to define the natural behavior (in causing cancers and leukemia in laboratory and feral mice) of C type RNA viruses.

In addition, Dr. Rhim is currently embarked on a new approach designed to concentrate large amounts of tissue culture grown murine sarcoma and leukemia viruses to be used for various diagnostic purposes by the VCB, other collaborating NCI groups and NIAID. The observations that certain in vitro grown leukemia viruses may produce transformation of rat and hamster embryo cells in vitro, and that the rat embryo cells may provide culture systems of choice for assaying many of the newly isolated mouse leukemia viruses in the COMuL test will be exploited in studies of the extra-host proclivities of these viruses.

Date Contract Initiated: Original Contract PH43-62-200 - 3-12-62
Present Contract PH43-68-705 - 2-1-68

Current Contract Level: $234,000

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-68-706; formerly PH43-63-81)

Title: Development, Growth and Characterization of Virus-Induced Tumor Cells

Contractor's Project Director: Aaron Freeman, Ph.D.

Project Officer (NCI): Robert J. Huebner, M.D.

Objectives: (1) To establish and characterize experimental cell lines derived from solid tumors and normal tissues of a number of species, the former to serve as sources of oncogenic viruses or of their genomes, the latter for isolation and propagation of cancer viruses and for measurement of viral transformation potential; (2) to study in tissue culture systems cells transformed in vivo and in vitro by the murine leukemia-sarcoma complex viruses; (3) to study viral-chemical co-carcinogenesis in tissue culture systems for both RNA and DNA transforming viruses.

Methods Employed: Dr. Freeman, the Project Director, has employed all of the standard tissue culture procedures. In addition, in view of the universal difficulty in growing various cancer cell lines, he has utilized or developed new experimental procedures for this purpose. These include grid cultures of organs and tissues and agar cultures. Dr. Freeman's group pioneered in developing in vitro transformation systems for determining the oncogenic behavior of human adenoviruses. His low calcium cultivation method led to the transformation of rat cells by the "non-oncogenic" adenovirus types 2, 5 and 6, and subsequently type 1 by Dr. McAllister.

Significance to Biomedical Research and the Program of the Institute: Since 1962 this contract, supervised by Dr. Freeman, has served as a basic support laboratory for our intramural cancer-virus research program. At the present time NCI Contracts 941, 705, 634, 697, and the intramural studies of Rowe, Hartley, Lewis, Hoggan, etc. are heavily dependent on the tissue culture reagents, cells and services provided by this contract, including the present effort to recover and/or rescue a sarcoma virus/genome from human sarcomas presently being supplied by a subcontract (NCI 941) but which will soon be supplied by an NCI contract with the University of Southern California. Dr. Freeman's low calcium transformation system led to the demonstration of group-specific T antigens and mRNA's common to four adenoviruses (types 1, 2, 5 and 6) previously listed as non-oncogenic, thus establishing a third group of potentially oncogenic adenoviruses. Since these viruses represent almost universal infections of infants and children and are most frequently found persisting in the adenoids and tonsils years later any possible influence they may have on the occurrence of human cancer must be determined.

Proposed Course: In addition to serving as a prime resource of cell lines derived from both tumor and normal tissue as required, the scope of this contract has been enlarged to incorporate a virology section. Studies of the many cell lines established from spontaneous, chemically-induced and virus-induced solid tumors of the mouse have led naturally to studies of the role of infectious and defective virus both alone and in combination with carcinogens in causing in vitro transformations of cells. This approach may be of most importance in attempts to transform human cells.

Cell lines of various nonlymphomatous solid tumors of humans which have been established in tissue cultures will be grown out in large amounts and will be studied by contractor and other collaborating laboratories for focus-forming infectious sarcoma virus, virus particles in the electron microscope, for defective sarcoma virus genomes, for virus-specific T antigens of the adenoviruses and mRNA of the human and animal adenoviruses.

Dr. Freeman's group has and will continue to furnish most of the numerous services required for providing special experimental and cancer cells for most of the in vitro studies in the VCB programs. Hundreds of cell lines from humans, cats, dogs, cows, monkeys, numerous strains of mice and rats are frozen in nitrogen and are available for use in all NCI intramural and contract operations. In 1969 a concerted effort will be made to establish human embryo cell lines derived from different ethnic and racial groups and hopefully also from inbred populations. The latter are needed in order to insure that human cells susceptible to postulated human sarcoma viruses will be available for use.

Date Contract Initiated: Original (NIAID PH43-63-81) - 11-16-62
Present (NCI PH43-68-706) - 2-1-68

Current Contract Level: $310,050

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-64-941)

Title: Project A - The Study of the Role of RNA Cancer and Leukemia Viruses in Animals and Humans.
Project B - Co-carcinogenesis--Viral-Chemical Carcinogenesis Studies.
Project C - Collection and Study of Leukemia and Sarcoma Materials.

Contractor's Project Directors: Project A - Padman S. Sarma, D.V.M.,Ph.D.
Project B - Howard J. Igel, M.D. (until 4-68)
Carrie Whitmire, Ph.D. (present Project Director)
Project C - Murray B. Gardner, M.D. (USC)
(Sub-contracts) Robert M. McAllister, M.D. (Childrens Hospital, Los Angeles)
Zenobia Holbrook (Orthopaedic Hospital, L.A.)

Project Officer (NCI): Robert J. Huebner, M.D.

PROJECT A

Objectives: Dr. Padman S. Sarma, Project Director, is concerned with an entirely new project, namely, adapting newly developed *in vitro* diagnostic methods to demonstrate and assay "C" type RNA viruses of the leukemia-sarcoma complex in laboratory rats, in cats, and in various avian species - particularly chickens, quail, and parakeets. He is also engaged in a collaborative study, with Dr. Samuel Baron of NIAID, of interferon effects on "C" type RNA viruses. The ultimate purposes of this project are two-fold: (1) to determine possible cross infection of humans intimately exposed to these animals and their leukemia viruses and (2) to learn more about the distribution and natural behavior of the "C" type RNA viruses.

Methods Employed: Contractor will use modifications of the COFAL and COMuL tests for avian and murine leukemia viruses (developed by Sarma *et al* and Hartley *et al*), as well as focus-forming and genome rescue tests for sarcoma (developed by Hartley *et al* and Huebner *et al*) in studies of the frequently naturally occurring nonlymphomatous and lymphomatous solid tumors of the three groups of animals mentioned above.

Major Findings: This is a new project. On previous projects, Dr. Sarma either authored or played a major role in the development and application of (1) the COFAL test; (2) the demonstration of soluble group-specific internal antigens of avian leukosis-sarcoma viruses in virus free non-producer Rous sarcomas of chickens, quail, hamsters and monkeys; (3) the transfer of the virus free Bryan Rous genome from hamster to chicken cells; and (4) recently, the development of an "interference" assay test for murine leukemia.

Specific studies carried over from previous projects include: (1) Effect of DEAE dextran on the growth of MSV in mouse embryo fibroblasts; (2) In vitro effects of mouse interferon on various murine leukemias and pseudotype murine sarcoma viruses; and double stranded RNA on Rous sarcoma virus and murine leukemia viruses; (3) Transfer of defective Rous sarcoma genome from hamster tumor cells to quail cells; and (4) Studies of a serial tissue culture line of a virus free Schmidt-Ruppin Rous sarcoma hamster tumor cell.

Specific findings in relation to above projects include: (1) DEAE dextran increases MSV yield from dextran-treated MEF and enhances MSV focus formation in mouse embryo fibroblasts (MEF), most noticeably with the Gross pseudotype MSV (GL-MSV); (2) preliminary results in MEF indicate that mouse serum interferon completely suppresses Friend leukemia (FL) virus and markedly suppresses Rauscher leukemia (RL) virus, and that the FL-MSV pseudotype is more sensitive to interferon effects than the RL-MSV; (3) Dr. Sarma has also demonstrated in vitro that purified interferon and double stranded RNA (Poly I.C.) suppressed the Bryan strain of Rous sarcoma virus in chick embryo fibroblasts (CEF) and the Friend virus pseudotype (FL-MSV) in mouse embryo fibroblasts (MEF). No effects have been observed in vivo but, as stated, experiments are still in the initial stages; and (4) preliminary results indicate that the Bryan strain Rous sarcoma virus genome can be rescued from virus-induced but virus-free hamster tumor cells by the use of quail embryo fibroblast cells and avian leukosis helper virus. No results are as yet available on the newer projects described in Objectives.

Significance to Biomedical Research and the Program of the Institute: All of the methods described above are in tissue culture systems. The real aim of these studies in domesticated animals is the development of tissue culture methods which ultimately will be applicable to studies of the etiology of solid tumors of man, a problem which it is clear will have to be studied entirely in in vitro systems. In addition, it is quite possible that leukemia and sarcoma viruses of pets and domestic animals will, under certain conditions, cross species lines sufficiently well to infect man himself. Dr. Sarma is working in close collaboration with other NCI contractors and collaborating groups, including two contract groups at the University of California (PH43-63-13 and PH43-65-609), Dr. Rickard at Cornell University (PH43-65-620), Dr. Kirsten of the University of Chicago, Dr. Ray Bryan of NCI, SVLP contract groups in Florida, and other contract groups within Microbiological Associates.

Proposed Course: Effective studies of viral induction of cancer in the rat, cat, parakeet and other avian species are entirely dependent on appropriate assay systems, and present emphasis is on the development and adaptation of effective *in vitro* tests for "C" type viruses in solid tumors as well as lymphomas of these animals.

PROJECT B

Objectives: This project, originally set up by Drs. Paul Kotin, Robert Huebner and Hans Falk, and supervised by Dr. Howard J. Igel (NCI resident Project Officer who resigned in March, 1968), will continue to be devoted to the development and performance of precise studies of viral-chemical co-carcinogenesis in C57Bl mice. As the new Project Officer, Dr. Robert J. Huebner has accepted responsibility for directing the research program, and Dr. Carrie Whitmire, an experienced virologist, will assume Project Director responsibility for the co-carcinogenesis experiments. Dr. Igel, however, will provide guidance and critical histopathological services under a consultancy arrangement, and Dr. Hans Falk has indicated his desire and willingness to collaborate with Dr. Huebner in future projects.

Major Findings: Working in a murine system previously presumed to be free of leukemia or other viruses, the C57Bl mouse, Drs. Igel, Huebner and Falk demonstrated that solid tumors induced by chemical carcinogens, either alone or in combination with the Kaplan leukemia (KL) virus, frequently contained the specific viral antigens of the murine leukemia-sarcoma group. He further demonstrated that the KL virus as well as wild leukemia isolates of the C57Bl mouse served effectively as "helper" viruses in rescuing the defective MSV genome, thus producing KL-MSV pseudotype virus. Tumor induction experiments with the KL-MSV pseudotype sarcoma virus was localized at the site of the intramuscular tumor which proved to be a typical MSV fibrosarcoma. Dr. Igel's repeated efforts to demonstrate the replication of KLV in tissue culture were finally successful with the development of broadly reactive MSV rat CF antiserum and a high passage cell line of C57Bl/6 mouse embryo. Further, Drs. Igel, Hartley and Huebner ascertained that previous inability to demonstrate KLV replication *in vitro* was due to its unique specificity for the host cell rather than to an absolute defectiveness of the virus.

Co-carcinogenesis experiments with (1) influenza virus and diethylnitrosamine, and (2) mouse adenovirus and dimethylnitrosamine did not indicate synergism between virus and chemical. However, synergism was demonstrated between KLV and 3-methylcholanthrene. Results as determined by tumor induction and positive CF antigen *in vitro* suggested that the development of KLV serum antibody in methylcholanthrene-treated mice may be due to activation of mouse leukemia virus.

Significance to Biomedical Research and the Program of the Institute: These findings have conceptual as well as scientific importance. The occult behavior of the KL and other C57Bl leukemia viruses provides a model that mimics the human cancer situation and therefore may prove to be valuable for studying various degrees of leukemia virus gene expression. Furthermore, the demonstration of specific leukemia-sarcoma viral antigens in chemically induced tumors of laboratory mice suggests a re-examination of current theories on the mechanisms of chemo-carcinogenesis. Like the leukemogenic effects of radiation, leukemia and cancer induction in mice by chemicals may be due to release of switched-off viral genes rather than their effect on cell genes. This observation poses perhaps the most important question that can be asked concerning the mechanism of chemical oncogenesis in the much used oncogenic virus-ridden mouse assay system.

Proposed Course: Project B will continue as outlined above. However, major emphasis will be the determination of the role of covert (phenotypically unexpressed) "C" type viral genes in chemical carcinogenesis. In cooperation with Dr. Freeman (NCI Contract PH43-68-706), co-carcinogenesis between chemicals and DNA tumor viruses (chiefly human adenoviruses) are being explored.

PROJECT C

Objectives: This portion of the contract includes three subcontracts with the University of Southern California and two hospitals in the Los Angeles area to provide nonlymphomatous sarcoma materials from children and adults for study by the Viral Carcinogenesis Branch and investigators on research contracts supervised by the Branch, including the program on 941. The chief purpose is to look for evidence of oncogenic genomes in human solid tumor cells using the various new techniques for demonstrating non-infectious fingerprints of suspected viruses as well as for the viruses themselves. In order to explore the possible application of the genome rescue procedures developed in the avian and murine systems, fresh specimens from human leukemic patients are also being collected to be used as "helpers."

Methods Employed: Most of the professional personnel assisting in the work of Project C are physicians, chiefly orthopaedic surgeons or pathologists. Together they have access to many of the sarcomas and leukemia in Southern California and surrounding areas with a population estimated at 15 million. Laboratory facilities are available at both Childrens Hospital and Orthopaedic Hospital for on-site processing of fresh specimens and for considerable amounts of investigation as well. Dr. McAllister, Project Director at Childrens Hospital, who has been engaged in collaborative studies with the Laboratory of Viral Diseases for the past several years, and Miss Zenobia Holbrook at Orthopaedic Hospital, have been growing fresh materials in tissue culture for genome rescue attempts using as "helper" human leukemic sera. Dr. McAllister in addition is engaged in studies of transformation with certain human adenoviruses. He is also attempting to obtain clones of malignant sarcoma cells in tissue culture using the agar overlay technique; and to rescue a sarcoma genome from human sarcoma cells grown in media containing human leukemic sera.

Major Findings: Project C has provided an unprecedented number of human leukemia and sarcoma materials to date. Many tissue culture cell lines have been established both in Los Angeles and at collaborating intramural and contract laboratories in Bethesda and St. Louis.

Significance to Biomedical Research and the Program of the Institute: In vitro methodology in experimental model systems have provided the technological and conceptual keys to many human virus disease problems. In the case of cancer-virus research, experience in experimental systems has demonstrated the absolute necessity for fresh tumor materials for use in all available study systems (focus-forming tests for avian and murine sarcoma, the COFAL and COMuL tests for corresponding leukemia viruses, genome rescue, viral interference and mRNA hybridization tests). Because of the specialized nature of the hospitals and personnel on this project, and the very populous area feeding into these hospitals and services, this project has provided and will continue to provide an invaluable resource not only to the VCB but to other parts of NCI's Viral Oncology Program.

Date Contract Initiated: 6-26-64

Current Contract Level: $697,030

LIFE SCIENCES, INC. (Formerly GERMFREE PRODUCTS, INC.) (PH43-68-711)

Title: Production of Germfree and Specific Pathogen Free Animals

Contractor's Project Director: Dr. Wendall M. Farrow

Project Officers (NCI): Mr. John P. Kvedar
Dr. W. Ray Bryan

Objectives:

(1) To maintain foundation colonies of germfree animals of species and strains required for program as designated by the Project Officers.
(2) To produce animals for research, both germfree and specific pathogen free (SPF) derived therefrom. The latter are maintained under environmentally controlled conditions which preclude intercurrent infection by pathogenic microorganisms or infestation by parasites, and are referred to as "reagent grade" hosts (RG-SPF).
(3) To transport or ship such animals to SVLP participants in completely germfree equipment as designated by program management through the Project Officers.

Major Findings:

(1) Germfree colonies of Balb/c and Alb/2 mice from a previous contract (with the same contractor) that had been completed were transferred to this recently initiated new contract. These are maintained as foundation colonies which furnish breeders for further expansion within germfree isolators for supplying germfree animals for research under other SVLP programs.

(2) Germfree quail are being produced and supplied regularly to other SVLP users as necessary for present programs.
(3) A RG-SPF colony of quail, which will be used in much larger numbers than germfree quail, is being built up with birds hatched germfree but transferred to GR-SPF facilities at an early age (a few weeks old).
(4) The similar production of GR-SPF chickens from a leukosis-virus-free stock (Luginbuhl) has been initiated and animals for experimentation should be available within a few weeks.
(5) Time-bred pregnant germfree females of two inbred lines of mice are being supplied regularly to another SVLP contractor for developmental research on ridding these lines of vertically transmitted viral agents (see contract PH43-65-95).
(6) A hamster colony has been established within environmentally controlled facilities and is being built up for eventually supplying time-bred pregnant females to another contractor for developmental research on efforts to establish a germfree line of hamsters. Germfree hamsters have not yet been successfully developed and are not now available for research.
(7) Surface-sterilized eggs from the RG-SPF quail colony are now being supplied in limited numbers, and with the full development of the colony will be supplied to SVLP participants at a rate of 500 per week.

Proposed Course: This service type contract for the production of germfree and reagent grade SPF animals will be continued, with the flexibility of being reoriented as rapidly as possible to meet changing needs of SVLP activities as they occur.

Date Contract Initiated: 2/8/68

Current Contract Level: $198,000*

* Yearly rate; initial funding was for a nine-month period.

Microbiological Associates, Inc. (PH43-66-914)

Title: Establish and Operate a BALB/c Mouse Colony

Contractor's Project Director: Mr. Wilbur Athey

Project Officer: Mr. Samuel M. Poiley

Objectives: The provision of BALB/cAn mice for laboratory investigations sponsored by SVLP, Etiology, NCI. The major portion of the mice are supplied for bio-assay studies supported by Contract No. PH43-67-697. Lesser quantities are furnished to Contract No. PH 43-65-1033, and to NCI in-house laboratories.

This project is self-sustaining in that the contractor maintains pedigreed foundation and expansion colonies, which produce the breeding animals for a large-scale production colony. Breeding mice are used to produce F_1 offspring.

The workscope of this contract is defined as the maximum number of mice of various categories that can be produced in a total of 5,000 cages. Specifications for categories are generally based upon age, weight, sex distribution, suckling litters, littermates or pregnants.

Methodology employed is based upon the specific protocol devised for this project, and the Institute of Laboratory Animal Resources Standards for the Breeding, Care and Maintenance of Laboratory Mice.

Major Findings: The contractor's Principal Investigators and the NCI staff users of these mice report that they are satisfactory for their purposes.

The contractor has demonstrated excellent cooperation in achieving production goals and supplying mice in the quantities requested by the various program areas.

Significance to Biomedical Research and the Program of the Institute:

The BALB/cAn mice produced under the terms of this contract have served to significantly advance the project supported by Contract No. PH43-67-697. These bio-assay studies are of particular significance to the Special Virus Leukemia Program. The availability of limited numbers in excess to the prime requirement has been beneficial to intramural program areas.

Proposed Course: The workscope of this contract will be reduced during the renewal period due to a predicted decrease in the rate of use for these mice. The building space made available in this manner will be modified for the development of an isolator-dependent colony.

Pedigreed mice will be obtained from flexible film isolators, transferred in sterilized and filtered boxes to this facility. The husbandry practices for this production activity will be identical to those applied for barrier-type colonies. Weanlings will be used for a series of preliminary studies in order to determine whether the new animals could successfully replace the conventionally reared mice. If the results are satisfactory, the new colony will be advanced to full production status, and the old colony phased out.

Date Contract Initiated: June 15, 1966

Current Contract Level: $92,730.00

Simonsen Laboratories, Inc. (PH43-68-68)

Title: Establish and maintain a production colony of BALB/cAnCr mice within the confines of 2,500 cages.

Contractor's Project Director: Mr. Harry C. Simonsen

Project Officer: Mr. Samuel M. Poiley

Objectives: To develop and maintain a production colony of BALB/cAnCr mice at a level that requires 2,500 mouse cages. Breeding mice are provided by the Government. Husbandry practices employed are in accordance with current knowledge in this industry. The contractor will upgrade his methodology in accordance with advancements in the fields of Laboratory Animal Medicine and Animal Production.

Animals must be free of Pseudomonas aeruginosa, Salmonella spp., and chronic respiratory disease. The contractor submits body fluids, intestinal contents and tissue to designated diagnostic laboratories as requested by the Project Officer.

Animals are distributed to organizations engaged in testing and research programs sponsored by the Government in accord with instructions and specifications (as to age, sex distribution, weight, pregnant, etc.) issued by the Project Officer.

Major Findings: The mice supplied to VLL, Etiology, during this period have been of excellent quality and have been satisfactorily applied for ongoing studies. The contractor has demonstrated excellent cooperation by achieving programmed objectives.

Significance to Biomedical Research and the Program of the Institute:

The availability of these mice has served to enhance the studies performed by the VLL Section. The contractor's ability to provide these animals in accordance with precise specifications has served to eliminate disruptions in laboratory schedules if procurement problems had not been resolved. Staff investigators have expressed the opinion that this contract is of particular value in their studies.

Proposed Course: No change other than improvement in methods as advancements take place in this field.

Date Contract Initiated: October 23, 1967

Current Contract Level: $42,235.00

MELPAR, INC. (PH43-65-641)

Title: Cell Biology Research

Contractor's Project Director: John E. Verna, Ph.D.

Project Officer (NCI): Charles W. Boone, M.D., Ph.D.

Objectives: To provide research facilities for the Cell Biology Section, VCB, NCI, in support of the following programs: (1) Isolation of pure viable tumor cell fractions from disaggregated solid tumors: (2) isolation and purification of virus-induced tumor-specific transplantation antigens; (3) studies of cultured cells undergoing spontaneous and virus-induced neoplastic transformation; (4) elucidation of the difference between normal and transformation prone human cells; (5) genetic studies of SV40 virus mutants.

Major Findings: A system has been developed for isolating viable tumor cell fractions from solid tumors using density gradient centrifugation in special rotors. It is now being applied to transplantable animal tumors of various types. A method for isolating highly purified plasma membranes from HeLa cells has been completed and is now being applied to the isolation of virus-induced tumor-specific transplantation antigens from normal and neoplastic cell culture lines. The appearance of a new surface antigen in mouse cells after transformation by SV40 virus has been detected using a highly sensitive I^{125}/I^{131} paired radioantibody technique. The transformation of human diploid cells in culture by adenovirus has been demonstrated.

Significance to Biomedical Research and the Program of the Institute: The ability to isolate purified tumor cell fractions from disaggregated solid tumors provides a means for making quantitative comparisons between tumor cells and homologous normal cells. The isolation of virus-induced, tumor-specific transplantation antigens will allow studies of their structure and their therapeutic action in protecting against tumor cell challenge. The demonstration of adenovirus transformation of human diploid cells provides an avenue for investigating the possible mechanisms of carcinogenesis using a human virus and a human cell. Genetic studies of virus mutants can provide the means by which the chromosomes of oncogenic viruses are mapped.

Proposed Course: One more year at current level of activity. Following this, the activity will be reduced as the staff of the Cell Biology Section becomes established in new facilities now being prepared in Building 37 on the NIH campus.

Date Contract Initiated: May 25, 1965

Current Contract Level: $241,000

Microbiological Associates, Inc. (PH-43-66-887)

Title: Modification of Oncogenic Viruses and Disease Induction

Contractor's Project Director: Dr. M. Gomez

Project Officers (NCI): Dr. M. A. Chirigos, Dr. W. Hook,
Dr. W. Turner, Dr. P. Ebert, Dr. W. Woods

Objectives: Under the direction of NCI project officers in part-time residence, a laboratory is maintained to pursue three areas of interest: (1) To study the host-virus-cell interrelationships employing leukemogenic viruses, and neoplasms induced by these viruses; (2) To determine whether oncogenic virus replication or primary virus-induced leukemias or lymphomas can be modified by physical, chemical, biological, or immune mechanisms, or a combination of these; (3) To investigate biochemical alterations which may occur in tissues as a result of oncogenic virus infection or primary virus-induced leukemias or lymphomas.

Major Findings: The AKR lymphoma system established recently has been tested with several clinically active drugs. Complete regression of local tumor and hematological remission was achieved with combined drug therapy. Deaths which occurred were attributable to causes other than leukemia (thrombocytopenia or anemia). Treatment with homologous serum antibody was effective in reducing viremia and splenomegaly (Friend leukemia virus) and tumors (sarcoma virus) with a concomitant increase in survival time. Interferon, induced in vivo by a copolymer, resulted in long-term survivors which appear to be free of Friend leukemia virus disease.

BALB/c mouse anti-lymphocyte serum (ALS) prepared in rabbits has been tested for its ability to inhibit the RES mediated antibody synthesis. Regression of Moloney sarcoma virus (MSV) induced tumors, consistantly observed in adult animals, does not occur in animals which were treated with ALS. Leukemic animals are being treated with ALS to determine its therapeutic value.

A gel filtration method has been developed for purifying highly infectious and immunologically active virus from spleen and tumors. This method will be used for purifying viruses to be used in the preparation of a polyvalent vaccine.

Tumor incidence and death of animals infected with MSV has been significantly enhanced as the result of co-infection with leukemia virus (RLV, FLV, MLV). Cell-free tumor extracts have been passage 4 times without leukemia "helper" virus and have maintained their high oncogenic potency. This may represent an in vivo induced hybrid. The very low tumor incidence which occurs in animals infected with low concentrations of MSV has been significantly enhanced by co-infection with RLV. This observation may lead to an in vivo system for studying viral co-carcinogenesis.

Guaroa virus, an RNA virus, enhances the replication of leukemia viruses (FLV and RLV). Of particular significance is that Guaroa virus at (10^{-8}) enhances FLV replication. This observation is indicative of a virus-virus interaction occurring in vivo that is measurable.

The histological examination of changes occurring in regressing tumors (induced by MSV) supports the suggestion of an immunological response of the host to the tumor. Antibody has been found in serum taken from mice with regressed tumors.

Two enzymes important in the biosynthesis of heme have been extensively studied. Delta-aminolevulinic synthetase (ALA-synthetase) and ALA-dehydratase were found to be significantly elevated in FLV and RLV infected mice. This study shows that the erythrocytopoiesis induced by RLV and FLV can be monitored by a biochemical parameter. Lysozymes have been reported to be elevated in human monocytic and mono-myelocytic leukemia. Infection with MLV, RLV, and FLV resulted in significant increases in lysozyme activity in plasma. In search of an enzymatic marker for sarcoma virus, ATP-ase activity of a 10-fold concentrated viral pellet was found to be significantly elevated.

Significance to Biomedical Research and the Program of the Institute: This contract is in support of basic and developmental research underlying objectives of both Intramural and Special Virus-Leukemia Programs of NCI. The studies on factors which modify (interfere with, or enhance) host-virus-cell relationships are pertinent both to possible new approaches to therapy or prevention, and to the detection and propagation of leukemia-associated viruses present in subliminal infections.

Proposed Course: The effort on this contract will remain the same Each of the 3 areas described under objectives will be pursued an emphasis will be placed on any findings leading to prevention and/ or cure of animal leukemia or lymphoma.

Date Contract Initiated: 6/16/66

Current Contract Level: June 16, 1967 - June 15, 1968: $269,000

CONTRACT NARRATIVES
OFFICE OF THE SCIENTIFIC COORDINATOR
IN VIRAL ONCOLOGY, ETIOLOGY, NCI
Fiscal Year 1968

The following contracts represent programmed activities on viruses in relation to human and animal breast cancer, under the aegis of the Breast Cancer Task Force of the NCI. Although this Task Force falls administratively under General Laboratories and Clinics, the fiscal management of virological aspects of its programmed research is located in the Viral Oncology area of Etiology. The scientific management and coordination of the following contract activities are responsibilities of the Office of the Scientific Coordinator in Viral Oncology:

GEORGETOWN UNIVERSITY SCHOOL OF MEDICINE (PH 43-65-53)

Title: Human Breast Cancer Studies

Contractor's Project Director: Dr. William F. Feller

Project Officer (NCI): Dr. W. Ray Bryan

Objectives:

(1) To determine whether viral etiological agents are associated with human breast cancer.

(2) To further develop the mouse mammary tumor virus system as a laboratory model for guiding investigations on the human problem.

Major Findings:

(1) Human Studies (Joint studies in collaboration with Contract PH 43-67-1176; see also): In studies reported upon last year (FY 1967) a small "virus-like" particle was observed in ultracentrifuge fractions of milk from 7 of 43 normal women (16%), and in similar milk specimens from 2 of 4 women with breast cancer. Four additional milk specimens from women with breast cancer (previously operated upon for removal of one breast) have been studied during this report year. Three of them showed the same small "virus-like" particles, making a total of 5 out of the 8 (62%) studied to date.

Studies were also made on biopsies of breast cancer tissue from 28 patients. Small "virus-like" particles, identical to those observed in milk, were found in specimens from 12 of the cancer patients. The particles were in clusters extracellularly, as well as in vacuoles within cells. Whether the observed entity represents actual virus, and, if so, whether it is etiologically related to the neoplastic disease or is simply a passenger remain to be determined. The possibility also exists that it may be a secondary invader capable of influencing the clinical course of breast cancer.

The specimen procurement, processing and fixation, and the collection of clinical and pathological data were carried out under this contract. The thin section electron microscopic studies were carried out by the collaborating contractor. A joint report of these studies is in press (Feller, W. F., and Chopra, H. C.: A small virus-like particle observed in human breast cancer by means of electron microscopy. J.N.C.I., (June) 1968).

(2) Studies on Mouse Mammary Tumor Virus: The studies on the mouse mammary tumor virus (MTV) under this contract are collaborative with intramural NCI scientists as well as with another contractor (PH 43-66-458) who performs biological assays on the MTV. The contractor supervises a mouse dairy for the production of MTV, using techniques that he has developed, and purifies and concentrates the virus for studies with others. He is also engaged in efforts to improve methods for further purification, including the preservation of biological activity during the process. A joint report with Dr. W. T. Hall, NCI, on "Biophysical and bioassay studies on milk fractions of genetically similar agent-bearing and agent-free mouse substrains" was published (W. T. Hall and W. F. Feller: J.N.C.I. 39: 1155-1185, 1967). Although there was not an absolute correspondence between strain tumor incidence and MTV particle quantity in the milk, nonetheless, high-tumor-strain mice had many more particles than their syngenetic low-tumor-strain counterparts.

Significance to Biomedical Research and the Program of the Institute:

One of the major programs of the NCI concerns the study of viruses in relation to the causation of human cancer. The detection by electron microscopy of entities which could represent a virus, in frequent association with human breast cancer, justifies the further investigation of this human neoplastic disease from the virological approach. Even if the observed entity is not an etiological oncogenic agent, it could represent a secondary invading virus capable of exacerbating the clinical course of breast cancer and the elimination of which might contribute to better control of the disease.

Proposed Course:

Further studies will be made to determine: (1) relative frequency of association of the "virus-like" entity with breast cancer of different histopathological and biological types; and (2) whether the entity is a virus, and, if so, whether it is a known agent or one heretofore unknown.

Date Contract Initiated: 11/19/64

Current Annual Level: $38,900

CHARLES PFIZER COMPANY (PH 43-67-1176)

Title: Electron Microscopy Related to Viral Studies of Human and Animal Breast Cancer

Contractor's Project Director: Dr. J. J. Oleson
Principal Investigator: Dr. H. C. Chopra

Project Officer (NCI): Dr. W. Ray Bryan

Objectives:

This is a professional services contract established to provide electron microscopic monitoring and collaborative research in support of virological research on human and animal breast cancer conducted by other investigators and contractors participating in programs of the Breast Cancer Task Force of the NCI.

Major Findings: (See also narrative for Contract PH 43-65-53).

In collaboration with Dr. W. F. Feller of Georgetown University, electron microscopic studies have been made on thin sections of pellets from milk of normal women and women with breast cancer, as well as biopsies of breast cancer tissue from non-lactating patients. A small "virus-like" particle has been observed in 7 (16%) of 43 milk specimens from normal women, 5 (62%) of 8 milk specimens from lactating breast cancer patients, and 12 (43%) of 28 biopsies of breast cancer tissue, studied to date. Whether the observed entity is a virus and the significance of its more frequent association with breast cancer remain to be determined.

Comparable studies on breast cancers of rats in collaboration with BCTF participants have been initiated recently. Somewhat larger "virus-like" particles, resembling those associated with mouse breast cancer, have been observed in some specimens of rat breast cancer already examined.

Significance to Biomedical Research and the Program of the Institute:

See preceding contract (PH 43-65-53), with which this contract is collaborative, for significance of the human studies.

A viral etiological agent has long been known to be associated with naturally occurring breast cancer in high-cancer-strain inbred mice. It is of biomedical importance to determine whether breast cancer in animal species other than mice may also have a viral etiology. Biological methods have failed thus far to reveal a similar etiological agent in breast cancer of rats. If "virus-like" entities of the type observed in the initial limited studies are consistently found in association with rat breast cancer, a laboratory model system will become available for study, comparable in its investigative problems to those which now exist for human breast cancer.

Proposed Course:

Studies on human breast cancer. The contractor will collaborate with additional participants in the BCTF program to study breast cancer specimens from other geographical areas and population groups (e.g., high- and low-cancer families, premenopausal and postmenopausal women, etc.).

Studies on breast cancer of rats and other animals. These studies have only recently been initiated and will be continued without change.

Date Contract Initiated: 6/28/67

Current Annual Level: $121,979

MELPAR, INC. (PH 43-66-458)

Title: Bioassay of Mammary Tumor Virus

Contractor's Project Director: Dr. John E. Verna

Project Officer (NCI): Dr. William T. Hall

Objectives:

(1) This is primarily a service contract for carrying out bioassays of mouse mammary tumor virus (MTV) activity on milk, tissue extracts and other specimens, or fractions thereof, for scientists participating in program activities under the aegis of the Breast Cancer Task Force of the NCI.

(2) Developmental research is performed in attempts to improve the assay procedure, which is based upon the hyperplastic-alveolar-nodule (HAN) response in hormone treated mice developed by Nandi.

Major Findings:

Previous work under this contract verified the HAN response as a reliable indicator of biological activity of the MTV as reported by Nandi, and provided preliminary dose-response data which were used by Dr. John J. Gart (NCI) as a basis for devising experimental designs for quantitative bioassays involving this response.

The major effort during this report year has been directed toward defining the quantitative parameters of the dose-response relationship, the testing of designs for quantitative assays including the identification of variables which can be controlled through balanced designs, and determinations of relative potencies between standard preparations of MTV prepared by different participating investigators and which will be used by them as reference standards in further quantitative assays. The comprehensive quantitative biological studies also include experiments to identify the factor (or factors) responsible for the "reversal phenomenon" i.e., a weaker response and a reversed regression at the strongest dose (lowest dilution) levels of the dose-response curve, which has long been known to be a complicating factor in investigations on the MTV. These studies are being conducted by intramural NCI staff (Doctors John J. Gart, William T. Hall and W. Ray Bryan) in collaboration with Dr. S. Nandi of the University of California who developed and introduced the HAN indicator reaction. Although 5 1/2 months are required for assays involving this reaction, this is only 1/3 of the time required for the only other available criterion of biological activity of the MTV, namely tumor development. Because of the long period required for bioassay, results are not yet available for most of the studies initiated during this report yea

Significance to Biomedical Research and the Program of the Institute:

Although the MTV has been known since 1936, quantitative biological studies of its activity, including quantitative bioassays, have not, until now, been feasible. This is because the tumor response, the only previously available indicator of biological activity, appears only very late in life (12 months to end of life span) when animals are dying off in ever increasing numbers from "natural causes." The HAN response in hormone treated mice requires only 5 1/2 months, and bioassays involving it can therefore be completed before appreciable numbers of animals are lost due to death.

The 5 1/2 month period for assays is still impractically long for most laboratories because of the prohibitively large amount of space that would be required for animal holding in a continuing investigative program on the MTV.

This contract facility makes it possible for investigators to supplement their studies on the MTV with quantitative biological activity information which otherwise could not be obtained. Many studies of importance to BCTF objectives require this information.

Proposed Course:

It is planned to continue this service contract at essentially its present level of operation while the methodology is still under development.

Date Contract Initiated: 12/30/65

Current Annual Level: $126,000

SUMMARY REPORT
VIRAL CARCINOGENESIS BRANCH
July 1, 1967 - June 30, 1968

OFFICE OF THE CHIEF

In late 1967, the programs within the Viral Carcinogenesis Branch which provided resources and logistical support to virus cancer programs of the NCI (Viral Resources and Repository Section, Laboratory Animal Program, Information Unit) were shifted from the Branch to the Office of the Associate Scientific Director. During the year there has been a gradual transfer of some of the personnel from the Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases into the Branch. Part of the NCI-NIAID Collaborative Research Program will be incorporated into the Branch activities, and Dr. Robert Huebner, NIAID, will become Chief of the Branch.

The new Viral Carcinogenesis Branch is implementing and coordinating a research program on virus-host relationships in solid tumors caused by viruses of the DNA and RNA types -- towards the overall objective of control of virus-induced human cancers when such viruses are found. The Branch operates on the basic principle that the planning and execution of an effective cancer research program requires the integrated application of knowledge from many different fields of biology. There are sections of the Branch which represent disciplines at each level of biological integration: A Cell Biology Section; Ecology and Epidemiology Section; Solid Tumor-Virus Section; and a Molecular Biology Section. In addition, the administration of the Solid Tumor-Virus Segment resides in this office.

The NCI-NIAID Collaborative Research Program (reported separately in the past) consists of an integrated contract and intramural research program, and a detailed discussion of the DNA and RNA tumor virus studies is reported under the Solid Tumor-Virus Program Area. Additional laboratory studies on the DNA tumor viruses are reported under the Ecology and Epidemiology Section of this report. Also, the individual contract reports for the LVD-NIAID Viral Oncology studies are included in this report.

Contract narratives for the Solid Tumor-Virus Segment are listed with the Special-Virus Leukemia Program. Other contracts which are related to the activities of the Branch and administered by the Branch are listed under Program Management (Melpar, Inc., PH43-65-641; Microbiological Associates, Inc., PH43-64-941, PH43-68-705, PH43-68-706); Testing and Monitoring Segment (Microbiological Associates, Inc.; PH43-67-697B, Flow Laboratories, Inc., PH43-67-1135); and, Special Animal Leukemia Ecology Studies (Flow Laboratories, Inc., PH43-67-1396; Jackson Laboratory, PH43-67-744).

CELL BIOLOGY SECTION

The activities of the Cell Biology Section are organized into five research areas as follows: (1) Isolation of pure viable tumor cell fractions from disaggregated solid tumors. Procedures are continuing to be developed for the isolation of pure viable tumor cells from human tumors as well as mouse myelomas, melanomas, mast cell tumors, hepatomas, adrenal carcinomas, breast tumors and Ehrlich carcinomas. In relation to the method of density gradient centrifugation being used, a new rotor has been developed which markedly increases the resolution of whole cell separations. (2) Isolation and purification of virus-induced tumor-specific transplantation antigens. A method for the isolation of pure plasma membranes from HeLa cells has been completed. The isolation of plasma membranes from normal and transformed mouse and hamster cells is underway. (3) Spontaneous and virus-induced neoplastic transformation in vitro (mouse and hamster cells). In connection with the tumorigenicity testing required for this work, a unique method has been developed for the quantitative tumorigenicity assay of cultured cell lines using the anterior chamber of the mouse eye. (4) Elucidation of the difference between normal and "transformation prone" cells. A markedly increased susceptibility to transformation has been found with cells from individuals with Fanconi's anemia and Down's syndrome. Both of these genetic diseases are associated with a high incidence of spontaneous tumors. In addition, work has been completed on the development of a rapid assay for cellular sensitivity to viral transformation suitable for screening human populations. (5) Genetic studies of SV40 virus mutants. The preparation of SV40 virus mutants which have lost their ability to transform cells and to produce tumors, and the preparation of mutants which have high transforming ability, is being undertaken. With the aim of beginning a detailed genetic analysis of a small DNA tumor virus, mixed infection with SV40 mutants and search for recombinants is being performed.

Until the new Cell Biology Section laboratory is ready in Building 37, the intramural research of the Section (Individual Project Reports, Serial Numbers NCI 4900-4904) is being done in facilities provided by Melpar, Inc. (PH43-65-641) under contract to the National Cancer Institute.

ECOLOGY AND EPIDEMIOLOGY SECTION

Laboratory Studies of DNA Tumor Viruses -- Basic studies of adenovirus and SV40 oncogenesis continues to be focused on the significance of the virus-specific non-virion T or tumor antigens, the biology of adenovirus-SV40 hybrids and also on questions related to *in vitro* neoplastic transformation.

(1) T or Tumor Antigens -- Drs. Wallace Rowe and Andrew Lewis, LVD, NIAID, and their associates, using a sensitive FA test, demonstrated antibodies to adenovirus T-antigens in many children and adults; antibodies which occur no more frequently in cancer patients than in healthy matched controls. Since these antibodies have a long life span, they suggest that some adults (as do children in their adenoids and tonsils) may also harbor persistentadenovirus infections.

Drs. Raymond Gilden and Jerome Kern (Flow Laboratories, Inc., PH43-65-634) found that T-antigens could be selectively produced free of virion in two different ways. Cultivation of infected human cells maintained for 20 days at well controlled low temperature levels in the range of 23°C (after 4 hours at 37°C) led to the gradual accumulation of high titered T-antigen in the cells, while the viral protein antigens were held at very low or undetectable levels. Dr. Jay Levy, LVD, NIAID, working with Drs. Gilden and Kern found that hydroxyurea, an inhibitor of DNA synthesis, blocked virion but permitted T-antigen production; this latter finding proved to be the method finally selected for producing the T-antigens used for the serological studies of human cancer described under the Solid Tumor-Virus Program report.

Drs. Rapp and Zbar of NCI and Dr. Ariel Hollinshead and her associates at George Washington University (PH43-64-515) developed single and double cycle sephadex techniques for separating T-antigens from adenovirus infected cells which were sufficiently pure to offer opportunities for chemical analysis as well as for serological investigation. The latter studies are being conducted in Dr. Huebner's laboratory. Dr. Hollinshead's group demonstrated two distinct T-antigens with remarkable different molecular weights in the adenovirus type 12 infected cells.

These studies of the virus-specific tumor antigens of human viruses which cause cancer in laboratory animals are of considerable importance to questions concerning the possible roles of these viruses on the human cancer.

(2) The Biology of Adeno-SV40 Hybrids -- A large amount of effort has been devoted to characterizing clonal lines of adenovirus 2 - SV40 hybrid viruses in order to determine the

degree of genetic heterogeneity between different hybrid particles. The most striking finding during the past year was the detection of a non-defective hybrid (B-89) virus. Unlike all other hybrids, this virus does not require normal adenovirus as a helper, induces plaques in monkey cells, as well as human cells, on a one-hit basis, and is free of infectious non-hybrid adenovirus and SV40. This virus is also exceptional in not producing the T-antigen of SV40, although the presence of SV40 DNA in the virus has been proved by nucleic acid homology tests.

(3) In Vitro Transformation by SV40 and Human Adenoviruses -- Dr. George Todaro, Cell Biology Section, VCB, has demonstrated markedly increased sensitivity to SV40 transformation of human cell lines from children with genetic diseases such as Fanconi's anemia and Down's syndrome. Recent efforts to transform these "transformation prone" human cells with human adenoviruses appear to have succeeded also; if confirmed, this will represent an exciting new finding of importance to human oncogenesis.

Dr. Aaron Freeman and his associates, Microbiological Associates, Inc., (PH43-67-706) demonstrated that adenoviruses types 2, 5 and 6 produce transformation of rat cells grown in low calcium media. Shortly thereafter Dr. Robert McAllister of the Childrens Hospital Research Institute, Los Angeles, demonstrated the same property for adenovirus type 1. Since the cells transformed by all 4 serotypes were shown by Dr. Huebner to contain the same T or tumor antigen and by Dr. Green, St. Louis University (PH43-67-692), to contain identical virus-specific RNA's, these 4 adenoviruses now form a third, or "c" group, of oncogenic adenoviruses. These findings are extremely important since types 1, 2, 5 and 6 produce the most frequent adenovirus infections in young infants and represent serotypes isolable from as many as 75 to 80% of the adenoids and tonsils removed from children at surgery.

(4) Adeno-Associated Viruses -- Drs. Hoggan and Rowe, LVD, showed that types 2 and 3 of the newly discovered adeno-associated viruses (AAV's) produce infections in children. Studies in Junior Village in Washington, D.C. indicated that these infections occurred most frequently during periods when adenoviruses are most prevalent, however, the data are as yet insufficient to determine if these defective viruses influence the occurrence or severity of disease.

SOLID TUMOR-VIRUS SECTION

This Section was only recently established and the intramural research is being conducted at Flow Laboratories, Inc. (PH43-67-1135) until the new facilities in Building 37 are available. Currently, the research investigations are concerned with the isolation and identification of specific non-virion (early) antigens from cells infected with herpesviruses and determining if a relationship exists between the known herpesviruses and the herpes-like particles recovered from cultures of various leukemic and other cells.

Dr. Berge Hampar, NCI, and his associates have shown that early and late rabbit 7S and 19S antibodies to herpesviruses differ (1) in their ability to neutralize isologous virus under various conditions, (2) in their ability to differentiate between intratypic virus antigenic variants, and (3) in their ability to cross-react with heterologous herpesviruses. The findings are of interest both in terms of defining the properties of different antibody classes and in characterizing the cross-reactions between the known herpesviruses and the herpes-like particles (Individual Project Report, Serial Number NCI-4922).

Serial No. NCI/NIAID 68 A
1. Office of the Chief, VCB
2. Viral Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967-June 30, 1968

Title: Field Studies of the Role of Viruses as Causes of Human Cancer.

Previous Serial Number: Same

Principal Investigator(s): Drs. Robert J. Huebner, Wallace P. Rowe, Janet W. Hartley (LVD, NIAID); Drs. Andrew Lewis and James Duff (NCI).

Other Investigators: Mr. Horace Turner, Mr. William Lane, Mr. John Estes (LVD, NIAID); Drs. John Moloney, Howard Igel, M. Hatanaka, Herbert Rapp, Berton Zbar, Robert Depue, and Frank Rauscher (NCI).

Cooperating Units: Orthopaedic Hospital, Los Angeles (Sub-contract of NCI Contract PH43-64-941)
Dr. Vernon Luck
Dr. Herbert Stier
Dr. Peter Schwinn
Dr. David Monsen
Miss Zenobia Holbrook

Childrens Hospital, Los Angeles (Sub-contract of NCI Contract PH43-64-941)
Dr. Robert McAllister
Dr. Denman Hammond
Dr. Harry Wright
Miss Jean Filbert

USC Medical School, Los Angeles (Sub-contract of NCI Contract PH43-64-941)
Dr. Murray Gardner
Dr. Clayton Loosli
Dr. Bernard Hanes
Dr. Hugh Edmondson
Dr. Bernard Portnoy

Flow Laboratories, Inc. (NCI Contract PH43-65-634B)
Dr. Raymond Gilden
Dr. Jerome Kern
Mr. Theodore Beddow

Cooperating Units:
(continued)

Microbiological Associates, Inc. (NIAID Contract PH43-63-81, currently NCI PH43-68-706; NIAID Contract PH43-62-200, currently NCI PH43-68-705; NCI Contract PH43-64-941A)
Dr. Aaron Freeman
Dr. Johng Rhim
Dr. Padman Sarma

Naval Biological Laboratory (NCI Contract PH43-63-13)
Dr. Alexis Kniazeff

Baylor University
Dr. Joseph Melnick
Dr. Fred Rapp

M.D. Anderson Hospital (NCI Contract PH43-68-631)

George Washington University (NCI Contract PH43-64-515)
Dr. Ariel Hollinshead
Dr. T.C. Alford
Dr. S. Orozlan

St. Louis University (NCI Contract PH43-67-692)
Dr. Maurice Green
Dr. K. Fuginaga
Miss Magdalena Pina

Man Years:

Total:	7.5
Professional:	1.5
Other:	6.0

Project Description

Objectives: To conceive and coordinate comprehensive intramural and collaborative research aimed at the development of field programs designed to find answers to the following questions:

1. Are representatives of the 30 DNA viruses (adenoviruses, papovaviruses) which induce cancer in experimental animals also responsible for solid tumors?

2. Are "C" type RNA viruses, similar to those known to cause leukemias and solid tumors in chickens, mice, other rodents, cats and dogs, causes of the very similar cancers which occur in man?

3. Are any of the animal viruses known to cause cancer transmissible to man and/or epidemiologically associated with the incidence of human cancer?

4. Are low but long term exposures to known chemical and physical oncogens responsible for the significantly higher rates of cancer reported everywhere in densely populated metropolitan areas? It should be pointed out that virus infections and environmental carcinogens are known to be much more prevalent in urban than in rural environments; and there are available for study urban ecologies wherein environmental contaminations with known carcinogens exist in extraordinary amounts.

Methods Employed: In the search for evidence of DNA virus genes in human cancer, two highly correlated technical approaches were believed ready for application to the human cancer problem: serological tests for detecting adenovirus specific T or tumor antigens (see Serial No. 63 D); and biochemical tests for demonstrating adenovirus specific mRNA (see Contract Narrative, St. Louis University PH43-67-692). Similar procedures were also developed for DNA tumor viruses other than adenoviruses, including SV40 and polyoma viruses.

Two options were available: (1) to test for adenovirus specific T or tumor antigens in human tumor cells by complement fixation and/or fluorescent antibody tests; and (2) to test the sera of cancer patients for specific antibodies to T or tumor antigens representative of the three groups of oncogenic adenoviruses. Since production of purified T and tumor antigen had been achieved in several of the research contract laboratories, approach #2 was selected to be carried out in the shortest possible time.

The complement fixing non-virion T and tumor antigens representative of the 12 oncogenic adenoviruses which cause tumors in hamsters (groups A and B), or transform rat cells (group C) were produced in quantity by Drs. Gilden and Kern (Flow Laboratories, Contract PH43-65-634B); by Dr. Klaus Schell (Microbiological Associates, Contract PH43-62-200 and Contract PH43-64-941); by Dr. Ariel Hollinshead of George Washington University (Contract PH43-65-515); and by Drs. Herbert Rapp and Berton Zbar of NCI.

Purified adenovirus virion antigens were furnished by Dr. Maurice Green (St. Louis University Contract PH43-67-692).

Detection of "C" type RNA viruses and viral genomes in human solid tumors. Unlike the DNA viruses described above, RNA tumor viruses have not yet been demonstrated in humans. Thus our field studies of this group of viruses are mainly concerned with a) the acquisition of optimum human tumor materials for study in the laboratory and b) epidemiological information on the occurrence of those types of human cancer which most closely correspond to the types induced in chickens, mice, rats, cats and dogs by "C" type RNA viruses.

Direct attempts to isolate human sarcoma virus. The recent demonstration that "C" type viruses can cause various histological types of naturally occurring avian and murine sarcomas suggests that the direct test tube methods now available (focus formation and cell transformation) for detecting these viruses should be used in extensive and systematic attempts to find a human sarcoma virus. This simple direct approach is important because it has not yet been tried in systematic fashion, nor have the more recent sophisticated tools and observations been incorporated in such studies.

Tests for covert human leukemia viruses. Modifications of the tissue culture based COFAL and COMuL tests for isolation of avian and murine leukemia viruses are now being applied to the human sarcomas and leukemias. For example, only 10 isolates of murine leukemia virus had been made (all from neoplasms) prior to 1967; during 1967, using the COMuL test, we isolated hundreds of strains of murine leukemia from spontaneous and induced neoplasms, and from normal tissues of normal mice as well. Attempts to adapt these new in vitro methods to the detection of human leukemia viruses is of obvious importance since it bypasses the need for human volunteers or animal inoculations.

Attempts to rescue a defective sarcoma genome. Since human tumors have generally been negative for virus particles visible in EM and have also failed to yield detectable infectious virus, it is logical to assume that if sarcomas are caused by such a virus it is most likely to be present in a defective state comparable to the defective avian and murine sarcomas RSV(0) and MSV(0). Consequently the methods similar to those used for rescue of RSV(0) and MSV(0) will be tried with human sarcoma cells.

Use of combined animal and in vitro techniques. Both avian and murine sarcomas induce virus free sarcomas in hamsters and rats; yet they yield virus specific CF antigens and antibodies which provided the basis for the in vitro COFAL and COMuL tests for the leukemia viruses in these classes of animals. The possibility that human sarcomas may also contain a similar antigen must be tested.

Combined biochemical and biophysical methods for detection of integrated "C" type RNA virus genes in human leukemia and sarcomas. Recently a Viral Carcinogenesis Branch scientist found that RNA viruses stimulate DNA synthesis in infected and transformed cells. Others have shown that when virus induced but virus free sarcomas are pulse labeled with radioactive materials that a virus specific RNA can be separated from the cellular RNA. These procedures, combined with various hybridization and homology procedures used for the DNA virus (see above), may prove important for identifying a leukemia-sarcoma virus RNA in human neoplasms.

Field studies of human cancer as a virus disease. Studies of the behavior of animal cancer viruses in model systems make it clear that conventional searches for conventional manifestations of conventional infectious agents must be replaced by the newer concepts, approaches, and laboratory techniques described above if one hopes to mount a search for human cancer viruses. Similarly, studies of the natural occurrence of virus-induced tumors in chickens and mice suggest that the new laboratory approaches and methods must be applied in our projected comprehensive field research program designed not only to provide large numbers of well defined human leukemias and sarcomas for laboratory study, but designed also to furnish well-controlle epidemiological and ecological observations on the occurrence of such cancers.

Such a study program is important because field observations not only supplement the laboratory effort but provide reinforcement, confirmation and occasionally redirection of the latter.

Major Findings: Studies of RNA viruses in relation to human cancer have reached the staging level in FY 1967; human sarcoma and lymphomatous materials were collected in quantity for study but definitive results are not yet available. (See Project Reports 68B, 68D, and 71D; and Contract Narratives NIAID #81 and NCI #941.)

Serological tests of human cancer patients and controls. Twenty or more sera representative of the major types of human cancer, and comparable control sera were collected under Dr. Robert Depue's (NCI) supervision at the M.D. Anderson Hospital on contract PH43-68-631; thus 390 sera were finally made available for complement fixation (CF) and fluorescent antibody (FA) tests by several collaborating groups.

Dr. Huebner's group tested the sera by complement fixation (CF) versus T, tumor and purified viral antigens; Drs. Zbar and Rapp (NCI) tested them with the adenovirus type 7 T antigen. The results of the CF tests were being rechecked by Drs. R. Gilden and J. Kern (Flow Laboratories contract PH43-65-634B). Using the FA test, Drs. Fred Rapp and J. Melnick tested the 390 sera versus T antigens produced under the cover of cytosine arabinoside in monkey kidney cells, and Drs. John Riggs and Edwin Lennette tested them versus tumor antigens present in hamster and rat tumors induced by oncogenic adenoviruses representative of the three oncogenic groups of human adenoviruses.

The results on the 390 sera were in agreement and unequivocally negative. Less than 4% of all the sera showed reactions; thus there was complete agreement that 96% of the cancer patients and controls contained no antibodies to the virus specific T and virion antigens of the oncogenic adenoviruses. The few positives in the cancer group were matched by equal numbers in the control group. All the evidence indicated that these reactions were randomly distributed, nonspecific cellular isoantigens, a conclusion supported by the almost complete lack of agreement among the test groups concerning the "positive" specimens.

Significance to Biomedical Research and to the Program of the Institute:

A well-controlled large scale serological-epidemiological study has shown that human cancer patients do not show significant antibody responses to the T or tumor antigens representative of the known oncogenic human adenoviruses. Although this result is conclusive with respect to this statement, other more sensitive tests must still be utilized in similar well-controlled studies in order to rule adenoviruses out of consideration as causes of human cancer. See Objectives and Methods for significance of other projects.

Proposed Course: This is described above in descriptions of various individual projects.

Publications:

Freeman, A.E., Black, P.H., Vanderpool, E.Z., Henry, P.H., Austin, J.B., and Huebner, R.J.: Transformation of primary rat embryo cells by adenovirus type 2. Proc. Nat. Acad. Sci. 58:1205-1212, 1967.

Freeman, A.E., Vanderpool, E.A., Black, P.H., Turner, H.C., and Huebner, R.J.: Transformation of primary rat embryo cells by a weakly oncogenic adenovirus type 3. Nature 216:171-173, 1967.

Gilden, R.V., Beddow, T.G., and Huebner, R.J.: Production of high-titer antibody in serum and ascitic fluid of hamsters for a variety of virus-induced tumor antigens. Appl. Microbiol. 15:657-660, 1967.

Gilden, R.V., Kern, J., Beddow, T.G., and Huebner, R.J.: Bovine adenovirus type 3: Detection of specific tumor and T antigens. Virology 31:727-729, 196

Gilden, R.V., Beddow, T.G., Kern, J., Freeman, A.E., Martin, C.E., Turner, H.C McAllister, R.M., and Huebner, R.J.: The T and tumor antigens of adenovirus group C-infected and transformed cells. In press.

Gilden, R.V., Kern, J., Lee, Y.K., and Huebner, R.J.: Biological separation of adenovirus T and V antigen synthesis in KB cells. Proc. Soc. Exp. Biol. Med. 127:639-642, 1968.

Hollinshead, A., Alford, T.C., Oroszlan, S., Turner, H.C., and Huebner, R.J.: The separation and description of group A adenovirus-induced cellular antigens which react with hamster tumor antisera. Proc. Nat. Acad. Sci. 59:385-392, 1968.

Huebner, R.J.: Non-virion neoantigens in cells infected with and transformed by viruses. In: Specific Tumor Antigens. R.J.C. Harris, ed., Copenhagen, Munksgaard, 1967, pp.265-272.

Huebner, R .J.: Adenovirus-directed tumor and T antigens. In: Perspectives in Virology V. M. Pollard, ed. New York, Academic Press, 1967, pp.147-166.

Huebner, R.J.: The problem of oncogenicity of adenoviruses. PAHO International Conference, "Vaccines against Viral and Rickettsial Diseases of Man." PAHO, 1967, pp.73-80.

Levy, J.A., Huebner, R.J., Kern, J., and Gilden, R.V.: High titer T antigen with minimal amounts of structural antigen in adenovirus-infected cells treated with hydroxyurea. Nature 217:744-745, 1968.

McAllister, R.M., Reed, G., and Huebner, R.J.: Colonial growth in agar of cells derived from adenovirus-induced hamster tumors. J. Nat. Cancer Inst. 39:43-53, 1967.

Rhim, J.S., Schell, K., Turner, H.C., and Huebner, R.J.: Temperature dependence of the synthesis of adenovirus tumor and viral antigens. Proc. Soc. Exp. Biol. Med. 127:642-646, 1968.

Sarma, P.S., Vass, W., Huebner, R.J., Igel, H., Lane, W.T., and Turner, H.C.: Induction of tumors in hamsters with infectious canine hepatitis virus. Nature 215:293-294, 1967.

Schell, K., Rhim, J.S., Turner, H.C., and Huebner, R.J.: Adenovirus T antigen: Production by abortive infection of rabbit kidney cells. Proc. Soc. Exp. Biol. Med., in press.

Serial No. NCI/NIAID 68 B
1. Office of the Chief, V
2. Viral Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Title: Laboratory Studies of the Role of "C" Type RNA Viruses in Human and Animal Cancer.

Previous Serial Number: SAME

Principal Investigator(s): Drs. Robert J. Huebner, Wallace P. Rowe, and Janet W. Hartley (LVD, NIAID); Dr. Howard Igel (NCI); Dr. M. Hatanaka (Visiting Fellow).

Other Investigators and Cooperating Units:
LVD, NIAID (Mr. H. Turner, Mr. W. Lane, Mr. J. Est
NCI, Etiology Area and Special Virus Leukemia Program (Drs. Carl Baker, Frank Rauscher, and James Duff)
Microbiological Associates, Inc.
Contract PH43-68-706 (formerly NIAID 63-81) - Dr. Aaron Freeman; Contract PH43-68-705 (formerly NIAID 62-200) - Dr. Johng Rhim; Contract PH43-64-941 - Dr. Padman Sarma; Contract PH43-67-697 - Drs. R. Madison, G. Spahn, L. Rabstein, and R. Peters)
Flow Laboratories, Inc.
Contract PH43-65-634 - Drs. Raymond Gilden and Jerome Kern
Jackson Memorial Laboratory
Contract PH43-67-744 - Drs. Hans Meier and David Myers
Cornell University - Dr. Charles Rickard
Univ. of Pennsylvania - Drs. R. Marshak, R. Dutcher, and R. Brodey
Sloan-Kettering Inst. for Cancer Res. - Dr. Lloyd
Argonne National Laboratory - Dr. Miriam Finkel

Man Years:

Total:	16.2
Professional:	2.2
Other:	14.0

Project Description

Objectives: The primary objective of the VCB-LVD studies of the "C" type RNA viruses, which are natural causes of "solid tumors" as well as of leukemias in mice and chickens, is to further develop and to adapt new in vitro tests for these viruses to find out if similar neoplasms of man and his domestic animals are also caused by such viruses.

A concerted effort to study viruses in relation to solid tumors in the human is now believed feasible because the application in FY 1967 of newer in vitro techniques to studies of the natural behavior of murine leukemia-sarcoma viruses in natural and heterologous hosts have led to entirely new concepts, new approaches concerning modes of RNA viral carcinogenesis.

The newer techniques are also being utilized to help study the roles of "C" type RNA viruses in the solid tumors and leukemias of rats, cats and dogs and certain common avian species in addition to chickens.

Cooperative efforts continued with the Chemical Carcinogenesis Branch of NCI (Drs. Falk and Igel) to determine the role of RNA viruses in chemical inductions of cancer and leukemia in laboratory mice.

Methods Employed: Most of our efforts in FY 1968 were focused on the development of comprehensive collaborative programs to determine the natural history of the murine leukemia-sarcoma complex viruses and the extent to which natural infection is expressed as clinical leukemia, sarcoma or other solid tumors.

The techniques used were the in vitro procedures developed during the past 2 years by Huebner, Hartley and Rowe: (1) the focus forming (FF) test for sarcoma virus; (2) the COMuL test for the leukemia viruses; (3) the tissue culture test for neutralizing antibodies; (4) the complement-fixation (CF) test for virus specific gs antigen; (5) induction of virus free sarcomas in hamsters and rats with various avian, murine and human sarcomas; and (6) the genome rescue test for defective sarcoma virus.

Several laboratories were established to apply these laboratory procedures to field studies of animals carrying naturally occurring solid tumors and also in tissues of normal animals. The large mouse populations (including geriatric groups) of many strains were placed under intensive surveillance at the Jackson Memorial Laboratory (NCI Contract PH43-67-744) and the Walkersville facility of Microbiological Associates, Inc. (NCI Contract PH43-67-697). Tests for leukemia and sarcoma viruses in tumor and normal tissues were carried out by Dr. Johng Rhim, Microbiological Associates, Inc. (NCI Contract PH43-68-705) and Dr. Janet Hartley of LVD, NIAID. Tests for the presence and prevalence of the virus specific group specific (gs) antigens of the murine tumor viruses were performed by M-. Turner of LVD.

Dr. Gilden and his associates of Flow Laboratories Inc. (NCI Contract PH43-67-1396), with Drs. Rickard of Cornell University and Brodey of the University of Pennsylvania began attempts to isolate canine sarcoma and lymphoma viruses in tissue cultures. Dr. Sarma of MAI (NCI Contract PH43-64-941) began similar efforts to establish rat, cat, parakeet and quail leukemia viruses in tissue cultures.

Major Findings: The results of many studies carried out on contracts are described separately (see contract narratives).

Prevalence of leukemia-sarcoma "C" type viruses. A number of discoveries were made as follows: "C" type RNA viruses belonging to the leukemia-sarcoma group were found to rank among the most prevalent viruses of mice. (See NCI/NIAID Project Report 71 D and contract narratives for Jackson Memorial Laboratories and Microbiological Associates, Inc.). Several hundred wild strains were isolated from normal mice as well as tumored mice. All wild strains which were serotyped belonged to the Gross (G+) serological group as distinguished from the Friend-Moloney-Rauscher (FMR) serological group. Numerous "high" and "low" incidence mouse strains yielded "leukemia" viruses of the same serotype.

The properties of the "C" type murine viruses. The myxovirus-like properties of the "C" type murine leukemia-sarcoma viruses (MuLV and MSV) were confirmed. Like their avian counterparts, these viruses were shown to have an internal soluble protein coat best characterized as the group specific (gs) complement fixing antigen common to all of the known "C" type RNA viruses of the mouse, and an outer lipoprotein envelope best characterized as the type specific envelope antigen, which is distinct for the different serotypes.

The gs antigen demonstrated by Geering et al. to be released from the virus particle by ether treatment was shown to be like the avian gs antigen in its sedimentation properties; i.e., much lighter than the virus particle. Since it is a common antigen in all naturally occurring leukemia and sarcoma viruses, it provided a simple antigenic basis for the wide usefulness of the COMuL test.

Horizontal spread of leukemia virus. (Airborne?) At the Jackson Memorial Laboratory, 100 leukemia free but leukemia susceptible SWR strain mice were exposed to possible leukemia virus infection at 6 months of age by placing the SWR mice in open cages adjacent to cages containing naturally infected AKR and C58 mice of the same age. Between 40 to 100 days later, 4 of the SWR mice developed leukemia and their spleens and lymph nodes were shown to contain the virus specific gs antigen. Leukemia had not previously been found at Jackson Laboratories in 1500 SWR mice observed intensively until death. Similarly, a number of C57Bl six-month old mice, following the same exposure, developed CF antigen in their spleens, an observation not made previously in the normal mice of this strain. Since none of the mice were able to make intimate physical contact, the apparent spread of leukemia is presumed to have been airborne. Since Dr. Hellman and his collaborators at

Ohio State have demonstrated airborne infection of mice by aerosolized Rauscher virus, and we (Annual Report FY 1967) demonstrated MSV and MuLV in urine, saliva and feces of infected mice, horizontal airborne spread would not seem unlikely.

Naturally occurring MSV virus - nondefective (competent) MSV. The original Moloney and Harvey sarcoma viruses (M-MSV and H-MSV) were shown by Hartley, Rowe and Huebner to also contain excess amounts of nonsarcomatous leukemia virus. The M-MSV often required a 2-hit mechanism for replication. Recently we studied the FBJ "virus"--an osteosarcoma inducing virus--isolated by Dr. Miriam Finkel of the Argonne National Laboratory in 1965 from a bone tumor which occurred spontaneously in a CFI mouse. We have confirmed Finkel's observations and extended them by concentrating and purifying virus so that it now produces bone sarcomas locally within a few days after intramuscular injection of newborn CFI and NIH Swiss mice.

We have shown that the FBJ sarcoma virus is serologically identical and in other ways similar to "C" type mouse leukemia virus which we have found to be highly prevalent in normal CFI mice. The naturally occurring FBJ osteosarcoma virus, like the avian osteosarcoma and fibrosarcoma virus described by Rous (1911-1914), suggests that osteosarcoma and related muscle and fibrous tissue sarcomas can occur independently as competent viruses which is important because it suggests that direct attempts to isolate such viruses in the newly developed in vitro tissue culture systems should be tried.

Studies of "hidden" RNA virus genomes in spontaneous and chemically induced murine cancer; "switch on, switch off" mechanism. The discovery that avian sarcoma viruses were frequently defective, being incapable of replicating in the sarcoma cell and requiring a competent leukemia virus helper for infectiousness, provided a wholly new and unique view of the avian tumor viruses. When the Moloney sarcoma virus was also shown by LVD scientists to be partially defective in induced sarcomas of the mouse and the rat, and completely defective in sarcomas induced in hamsters, and that this lost infectiousness could be restored by a competent mouse leukemia virus, it became obvious that the MSV viruses were pathogenic mutants of leukemia viruses which were able to cause tumors in the absence of demonstrable replication of infectious virus.

Recently Drs. Hartley, Igel and Huebner, using new techniques, demonstrated that most of such lymphomas have a "C" type virus containing the gs antigen of the murine leukemias.

Also, in a long term collaborative study using the COMuL test, it was found that leukemia virus could be isolated in NIH Swiss cells, but not in BALB/c cells, from spleens of normal BALB/c mice. Virus was isolated with increasing frequency with age, approaching 80% at 20 months. However, when the BALB/c's developed leukemias and tumors late in life, viruses could no longer be isolated in NIH cells, but were readily isolated in BALB/c cells. Large numbers of isolation attempts have followed this paradoxical pattern, suggesting that while virus is frequently present, it is "switched off" with respect to the host (BALB/c) cells; when viewed in perspective perhaps a quite logical biological arrangement for a parasite to have with its host. Thus in the BALB/c cell the virus is a "switched off" MuLV(0) corresponding perhaps to the MSV(0) described by Ting; however for other mouse cells (NIH Swiss) it is "switched on" MuLV(+). However, when leukemia or solid tumors develop in the BALB/c mouse, the viruses isolated are now MuLV(+) for BALB/c cells and MuLV(0) for NIH - thus indicating a remarkable change in the indigenous BALB/c virus which takes place rather suddenly after oncogenic transformation occurs. This arrangement may be critical with respect to survival of the BALB/c strain and its leukemia virus as well; and is enormously interesting from a molecular biology point of view.

Another example of the "switched off - switched on" pattern is seen in the C57Bl mice, which yield no evidence of infectious virus or of gs antigen in their tissues as long as they remain normal; however, after spontaneous tumors occur or when tumors are induced by irradiation or chemicals, infectious virus can frequently be demonstrated in vivo, as reported by Kaplan; and in the COMuL test using a continuous line of C57Bl embryo cells, as reported recently by Igel and Huebner (in preparation). Although the amount of infectious virus demonstrated is very low in quantity, the tumor and splenic tissues often contain large amounts of gs antigen. The sudden "switching on" of demonstrable infectious virus at the time of spontaneous or chemically induced tumors suggests that the tumored mice contained a "switched off" MuLV(0) prior to tumorigenesis.

Studies of avian and murine sarcoma viruses. Pseudotype MSV's. Approximately 30 pseudotype mouse sarcoma viruses (MSV's) have been produced by using newly isolated wild strains of mouse leukemia viruses as helpers for the defective MSV hamster genome (Huebner, 1966). These viruses all induce almost identical fibrosarcomas in mice, showing that their pathogenic behavior is sarcoma genome dependent. However, their serological specificity and host-cell range in vivo and in vitro are determined by the leukemia virus envelope. Thus MSV's have been produced which have broad and narrow host ranges; some go only in one strain of mouse, others in many strains. These observations on mouse sarcoma which follow the same patterns observed for avian sarcoma are important because they increase significantly the possible pathogenic options availabe to the "C" type leukemia-sarcoma particles.

Demonstration of a "C" type virus in a rat tumor (Novikoff ascites). In 1967, in collaboration with Dr. Sarma (MAI NCI Contract 941), Drs. Huebner and Hartley initiated a systematic study of rats in an effort to detect either fully infectious MuLV(+) or, as seemed more likely, a silent MuLV(0) or MSV(0) viral genome.

Recently it was noted in Dr. Robert McAllister's group (Childrens Hospital of Los Angeles) that the Novikoff ascites rat tumor contained numerous "C" type particles. This tumor we found contained a CF antigen which reacted with a rat antisera having antibody to the Rauscher leukemia virus. However, there was no reaction with antisera prepared against the gs mouse antigen found in the Rauscher and all other murine leukemia and sarcoma viruses. Subsequent studies show that rats carrying the Kirsten and Novikoff tumors develop antibodies to Kirsten and Novikoff rat cell preparations but do not react well with the gs antigen of murine leukemia viruses. This suggests that the "C" type particle in the Novikoff rat tumor and perhaps also in the Kirsten rat sarcoma virus is a murine "C" type virus distinct from the mouse viruses.

Cooperative studies of virus-chemical carcinogenesis in the mouse. In previous studies of chemically induced tumors in C57Bl mice we failed to find evidence of murine leukemia-sarcoma virus activity. However in FY 1968, utilizing the modifications of the COMuL test and direct tests for the virus specific gs antigen of the leukemia-sarcoma virus, we succeeded in finding clearcut evidence for the presence of the C57Bl Kaplan leukemia type virus in virtually all such tumors. These observations, plus similar ones on other chemically induced tumors, are important because they raise questions concerning fundamental mechanisms of chemical carcinogenesis. The following question must be answered: do chemicals release or de-repress a neoplastic cell gene or do they release a hidden "C" type virus gene?

Significance to Biomedical Research and to the Program of the Institute: The newer isolations described above make it clear that the behavior patterns of the "C" type RNA viruses are almost identical in the classes of animals most studied for these agents. These viruses are extremely prevalent, and although frequently not expressed as frank cancer leukemia, they are present sufficiently often to account for many, if not most, cancers as well as leukemias in these animals. Should our field studies indicate that such viruses serve as the primary etiology for solid tumors as well as leukemia in the animals under study, similar viruses would have to be considered in the etiology of solid tumors in the human.

Proposed Course: The observations described suggest progressive adaptations of the new *in vitro* techniques to the solid cancer and leukemia problems of larger animals including man. Just as in our previous studies of avian and murine cancers, we plan to explore the role of naturally occurring "C" type RNA viruses in naturally occurring cancers in these "higher" species.

Since the question concerning "which gene" (cell or viral) is released by chemical carcinogenesis can probably be answered in virus defined mouse systems, efforts to find this answer will be given high priority during the next fiscal year.

Honors and Awards:

Member, Board of Scientific Advisors, Jane Coffin Childs Memorial Fund for Medical Research. (Yale University, New Haven, Conn.)

Howard Taylor Ricketts Award for 1968, for outstanding contributions to medicine. (University of Chicago, May 1968).

Guiteras Lecturer, 63rd Annual Meeting, American Urological Association, Inc. (Miami Beach, May 1968). Presentation: Cancer viewed as an infectious (viral) disease. Recent observations on human viruses that cause cancer in animals and on animal viruses that cause leukemia and sarcomas in their natural host.

Publications:

Hartley, J.W., Rowe, W.P., Capps, W.I., and Huebner, R.J.: Isolation of naturally occurring viruses of the murine leukemia virus group in tissue culture. In press.

Huebner, R.J.: *In vitro* methods for detection and assay of leukemia viruses. In: *Carcinogenesis: A Broad Critique*. Baltimore, Williams & Wilkins Co., 1967, pp.23-47.

Huebner, R.J.: The murine leukemia-sarcoma virus complex. Symposium on RNA viruses and neoplasia. *Proc. Nat. Acad. Sci*. 58:835-842, 1967.

Huebner, R.J.: Antigens of the leukemia-sarcoma virus complexes. In: *Proceedings of Perspectives in Leukemia, New Orleans*. Grune & Stratton, New York, 1968, pp.123-132.

Rhim, J.S., Williams, L.B., Jr., Huebner, R.J., and Turner, H.C.: Concentration by Diaflo ultrafiltration of murine leukemia and sarcoma viruses grown in tissue cultures. *Science*, in press.

Sarma, P.S., Cheong, M., Hartley, J.W., and Huebner, R.J.: A viral interference test for mouse leukemia viruses. *Virology* 33:180-184, 1967.

Invited Addresses:

Los Angeles Chapter, Western Orthopaedic Association, Los Angeles, California.

Seminar, Sloan-Kettering Research Institute; "Evidence for presence of leukemia-sarcoma virus genome in solid tumors of the rodents."

Presented paper, 95th Annual Meeting of the American Public Health Association, Inc., Miami Beach, Florida: "Immunology of Diseases in Public Health."

Invited speaker, Alpha Omega Alpha, College of Physicians & Surgeons of Columbia University, New York: "Studies of Viruses as Causes of Leukemia and Cancer."

Lecture, Georgetown University Medical School, Washington, D.C.: "New Developments in Cancer-Virus Research."

Presented paper, 45th Annual Meeting, American Epidemiological Society, Airlie House, Warrenton, Virginia: "Newer Observations in Cancer Viruses and Newer Approaches to Studies of Human Leukemia and Cancer."

Presented paper, Annual Meeting, American Academy of Allergy, Boston, Mass.: "Virus-Induced Changes in Cells."

Serial Number NCI/NIAID 68 H
1. Office of the Chief, VCB
2. Viral Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 - June 30, 1968

Title: Immunologic and Other Studies of Animal Leukemia Viruses

Previous Serial Number: Same

Principal Investigator: Dr. M. K. Cook

Other Investigators: Dr. R. J. Huebner, Miss Aurella Grochal (NCI)

Cooperating Units: Mr. William Jahnes (DBS), Division of Research Services, Los Angeles; Dr. B. Burmester, Dr. F. Siccardi and Dr. R. Witter, Regional Poultry Lab., USDA, East Lansing, Mich.; Dr. F. Wills, Univ. of Md.,; Dr. R. Bankowski, Univ. of California, Davis.

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives: Study of the biology and natural history of Type I (RIF-type) and Type III (RE-type) avian leukosis viruses and agents associated with Marek's disease.

Methods Employed: Immunofluorescence, complement-fixation, immunoelectrophoresis, agar-gel diffusion techniques, and neutralization tests are employed to define similarities and differences in the antigenic components of avian leukosis viruses. Column chromatography, electrophoresis, and sedimentation gradients were used to concentrate and partially purify these antigens. Tissue culture systems and standard animal inoculation procedures were employed.

Major Findings: An ether-chloroform sensitive COFAL negative, RIF negative, IUDR resistant filterable virus, 80 mμ in size, was found associated with chick embryo fibroblast tissue culture filtrates which produced Marek's disease in chickens. This virus (CSV) produced a syncytial type cytopathic effect in tissue cultures. Fluorescent antibody and neutralizing antibody to the agent were found in the sera of experimentally infected birds 55 days after inoculation. The possibility of the presence of other undemonstrated agents in the chick embryo fibroblast material and the limited number of animal experiments are recognized but present data suggest an association of CSV and Marek's disease. These experiments represent the first production of Marek's disease with 450 mμ filtrates of chick embryo fibroblast material.

CSV has been isolated from two field outbreaks of Marek's disease on the Eastern Shore.

The JM strain of Marek's disease has been established in chick embryo fibroblasts. A "round-cell" cytopathic effect was produced in 12-15 days after the initial inoculation. By the second passage, round-cell degeneration was noted as early as the fourth day after inoculation of tissue culture cells and supernatant fluid. Syncytial changes also were observed at this passage level. Passage of the JM agent has not been accomplished without the use of viable injected cells.

Further serologic surveys for RE virus, strain T, have revealed the presence of "T" virus antibody in chicken flocks on the Eastern Shore of Maryland. A possible antigenic relationship between the RE virus and CSV was suggested because fluorescent antibody for CSV was found in 56 percent of the birds with RE antibody. Fluorescent antibody for CSV has not been found in the absence of RE antibody.

The inhibition of Group I (RIF-type) avian leukosis by Group III (T-type avian leukosis) could not be traced to interferon production by the "T" virus.

The incidence of Group I (both A and B types) antibody in flocks maintained at one farm on the Eastern Shore of Maryland has shown a marked decrease in the past 18 months (from 55% to 6% antibody reaction).

Significance to Biomedical Research and the Program of the Institute: Avian leukosis viruses offer a model for the development of tools for the study of tumor viruses in other animals and man. They aid also in defining the natural history of oncogenesis. Marek's disease is a common lymphoproliferative disease affecting the central nervous system and peripheral nerves and occasionally other organs of the domestic chicken. It is very wide spread in the United States and the incidence of the disease has increased sharply in the past five years. Marek's disease agent(s) may contaminate viral vaccines prepared in chick embryo systems. The effect of such contamination is not known. Furthermore, methods for the detection of the agent are not defined. Identification of the agent(s) associated with Marek's disease and methods for their detection and control are of obvious importance to vaccine development programs for humans.

Proposed Course: Further studies of the association of CSV, JM virus and other agents isolated from Marek's disease will be made. Tests to isolate and identify such agents will be developed.

Attempts will be made to clarify the natural history of Group I and Group III avian leukosis infection by the use of fluorescent antibody techniques to demonstrate virus and antibody in leukemic flocks.

Publications:

Cook, M.K.: Cultivation of a filterable agent associated with Marek's disease. Submitted for publication.

Honors:

Invited speaker -- Avian leukosis RIF-free vaccine working conference, sponsored by U.S.D.A., Vet. Biologic Division, ARS.

Invited speaker -- Conference on Marek's disease, U.S.D.A., East Lansing, Michigan.

Serial Number NCI/NIAID 71 A
1. Office of the Chief, VCB
2. Viral Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967-June 30, 1968

Title: Studies of the Adenovirus-Associated Viruses (AAV).

Previous Serial Number: NIAID 71 A

Principal Investigator: Dr. M. David Hoggan

Other Investigators: Drs. Neil R. Blacklow (NCI) and
Wallace P. Rowe

Cooperating Units: Drs. James Rose and Aaron Shatkin, LBV, NIAID

Man Years:

Total:	3.6
Professional:	2.0
Other:	1.6

Project Description

Objectives: To study the biological, immunological, biophysical, biochemical and genetic properties of the adenovirus-associated viruses as well as study the natural history and the interrelationships between the various AAV and their adenovirus helpers.

Methods Employed: Standard virological procedures including complement-fixation, infectious titers in cell culture with helper virus, immunofluorescent assay, immunodiffusion, electron microscopic examination, density gradient purification, ultra-filtration concentration, and DNA-RNA *in vitro* homology techniques.

Major Findings: The AAV are small defective DNA viruses that will not replicate in cell culture in the absence of adenovirus. Based on complement-fixation reactions with antiserums made against various purified AAV preparations, AAV have been divided into 4 distinct types or groups. However, based on reactions between synthetic complementary RNA and DNA with various AAV, a number of the AAV strains *can* be further divided into substrains.

If early AAV serum is used for neutralization the serological data correspond to the homology data.

Although the natural history of AAV is not completely known, types 2 and 3 (and mixtures of these 2 types) have been isolated from throat and stool specimens from a group of institutionalized children. Further, between 50 and 70 percent of young adults have developed antibodies against types 1, 2 and 3. It therefore seems probable that these represent human strains. In contrast, AAV-4 can be repeatedly isolated from African green monkey kidney cell culture if competent adenovirus is added. In addition, some colonies of African green monkeys show as high as 67% incidence of neutralizing antibodies to AAV-4. These data strongly suggest that strains of AAV-4 are African green monkey in origin.

As mentioned above, AAV-2 and 3 have been isolated from children in the Junior Village population, and an intensive study of the children during an 8-week period has shown that AAV can spread in epidemic proportions. For example, 26% of 93 children were infected during this period with either AAV-2 or 3. Nearly all AAV isolates were accompanied by adenovirus isolates, suggesting a close relationship in vivo between AAV and adenovirus. No correlation between illness and isolation could be found.

A total of 6 strains of AAV have been purified in sufficient quantities to prepare DNA and synthetic complementary RNA for detailed study of their genetic relatedness. The amount of genetic relatedness between heterologous types has been estimated to be between 27-37%.

Using AAV-1 and AAV-2 it has been possible to detect messenger RNA in various infected cells only when such AAV infected cells are co-infected with adenovirus.

Attempts to initiate transformation with purified AAV preparations have not been successful even though preliminary attempts with heated crude preparations appeared to cause some morphological changes in cell cultures.

Significance to Biomedical Research and the Program of the Institute: The AAV's represent a completely unique phenomenon in animal virology - a virus group which appears to be absolutely dependent on another virus for its maintenance in nature. As such, determination of its natural history presents a challenging problem. Because of the pathogenic and potential oncogenic effects of adenoviruses, it is important to clarify the clinical importance of simultaneous AAV-adenovirus infections.

Proposed Course: Continued studies are in progress to further delineate the mechanisms of the block in AAV infection, the inhibitory action AAV has on adenovirus infection, and the reduction it causes in the oncogenic potential of adenovirus.

Publications:

Blacklow, N.R., Hoggan, M.D., and Rowe, W.P.: Immunofluorescent studies of the potentiation of an adenovirus-associated virus by adenovirus 7. J. Exp. Med. 125: 755-765, 1967.

Blacklow, N.R., Hoggan, M.D., and Rowe, W.P.: Isolation of adenovirus-associated viruses from man. P.N.A.S. U.S. 58: 1410-1415, 1967.

Blacklow, N.R., Hoggan, M.D., and Rowe, W.P.: Serological evidence for human infection with adenovirus-associated viruses. J.N.C.I. 40: 319, 1968.

Rowe, J.A., Hoggan, M.D., Koczot, F., and Shatkin, A.J.: Genetic relatedness studies with adenovirus-associated viruses. Fed. Proc. 27: 615, 1968.

Serial Number NCI/NIAID 71 E
1. Office of the Chief, VCB
2. Viral Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967-June 30, 1968

Title: Studies of Viral Antigens in Virus-Induced Tumors.

Previous Serial Number: Same

Principal Investigators: Drs. Wallace P. Rowe and Andrew M. Lewis (NCI)

Other Investigators: Drs. Michael N. Oxman and William H. Wiese and Robert J. Huebner

Cooperating Units: Drs. Patrick Henry and Myron Levin, NCI

Man Years:

Total:	7.5
Professional:	3.5
Other:	4.0

Project Description

Objectives: To study the function and significance of T-antigens of oncogenic DNA viruses, and to attempt to apply them as a tool in studies of cancer etiology; to study the biology of adenovirus-SV40 hybrid viruses.

Methods Employed: Tissue culture, fluorescent antibody, complement fixation, serologic epidemiology, and nuclei acid homology techniques.

Major Findings:

Adenovirus-SV40 hybrid viruses: A large amount of effort has been devoted to characterizing clonal lines of Ad. 2-SV40 hybrid viruses to determine the degree of genetic heterogeneity between different hybrid particles. The most striking finding is the detection of a non-defective hybrid (B-89) virus. Unlike all other hybrids, this virus does not require normal adenovirus as a helper, but induces plaques in monkey cells, as well as human cells, on a one-hit basis, and is free of infectious non-hybrid adenovirus and SV40. This virus is also exceptional in not producing the T-antigen of SV40, although the presence of SV40 DNA in the virus has been proved by nucleic acid homology tests.

In studies of other clonal lines of the Ad. 2 hybrid, it has been found that they fall into two distinct classes with respect to the frequency with which a cell infected with a hybrid particle yields infectious SV40: they either yield at high efficiency (about 100%) or low efficiency (about 0.01%). That is, there does not appear to be a continuous spectrum of yield frequencies, at least with this hybrid population. Subcloning of high and low frequency yielders has shown this to be a genetically stable marker. This means that with a high yielder, the hybrid particle can perpetuate itself as an adenovirus if infecting a cell along with a non-hybrid adenovirus, but under single-hit conditions will replicate as SV40; the low yielder must also contain the entire SV40 genome, since all hybrid clones have the capacity to yield free SV40, but the event (either in capsidation or in the singly infected cell) which determines that the SV40 pathway will be followed occurs only once in every 10,000 infections.

In studies of the formation of hybrids during joint replication of adenoviruses and SV40, it has been found that hybrids are generally formed within a single passage, but the frequency is so low (of the order of 1 hybrid per 10^6 adenovirus particles produced) that accurate quantitation is not possible with present techniques. In the course of this work a number of new Ad. 7 hybrids have been obtained which differ in significant respects from the standard E46 virus. In particular, most of the new Ad. 7 hybrids are capable of yielding free SV40; also, some appear to be linked to a different segment of the Ad. 7 genome, since they appear to induce different Ad. 7 T-antigens when transcapsidated to Ad. 2.

Highly encouraging results have been obtained in attempting to detect density differences of the hybrid and non-hybrid adenovirus particles by means of high resolution equilibrium density gradient techniques. $E46^+$ particles have been found consistently to peak one fraction lighter than the non-hybrid Ad. 7 particles in the mixture, while the Ad. 2 hybrid particles band 2 fractions lighter. Preliminary results suggest that one of the Ad. 2 hybrid clones is heavier than the non-hybrid, giving further evidence that the various clones are genetically distinct. Although the separation thus far achieved does not offer promise for preparative separations for biochemical and physical analysis, it should be useful for characterizing biologic activity.

Studies of antibodies to T-antigens: We reported last year that antibodies to adenovirus T-antigens were readily detectable in human sera by means of the indirect fluorescent antibody procedure. Two main lines of study have been conducted to further explore this finding. First, studies of the nature and natural history of these antibodies are essential for understanding their significance in seroepidemiologic studies. Dr. Wiese has found that the T-antibodies in two human sera are in the IgG fraction (these studies were done with reagents supplied by Dr. Julius Kasel, LCI) and that the reaction on the FA slide fixes complement. He has found that children at Junior Village show rises in T-antibody to various adenoviruses at different times, and is attempting to correlate these results with virus isolation data. In adults on whom serial sera spanning many years were available, the T antibody pattern was found to be stable, indicating either that these antibodies do not have the short lifespan seen with T antibodies in hamsters or that some adults are harboring foci of adenovirus antigens.

Controlled seroepidemiologic studies of antibodies to Ad. 2, 7, and 12 have been done with sera obtained for Dr. Huebner by Drs. Hollinshead and Alford. About 10 to 40% of sera showed FA reaction to T-antigens of the various adenoviruses, but there was no excess antibody frequency in the cancer groups as compared with matched controls.

The ability to make antibodies to T-antigens of "non-oncogenic" adenoviruses (Ad. 1, 2, and 5) by using tumors induced with their hybrids has proved very useful in identifying these antigens in rat cells transformed by the non-hybrid adenoviruses of these types. The lines of transformed cells obtained by Drs. Freeman, Huebner, and McAllister, have reacted with these antisera in both CF and FA tests, indicating the persistence and integration of the viral genome.

Detection of persistent viral genome by nucleic acid homology techniques: In the great majority of transformation by DNA viruses, the viral genome persists in the cells, as indicated by synthesis of T-antigen. However, some cell lines which showed signs of transformation after infection with SV40 or its DNA do not contain T-antigens; in the lines studied by Diamondopoulis and Enders, some lines showed SV40 surface(S) antigen without T-antigen, while others showed neither or both antigens. Drs. Oxman, Henry, and Levin have attempted to find SV40 mRNA in these lines by homology tests with SV40 DNA. Most important, they have developed the procedure to a point where the blank with normal cell RNA is almost zero, without markedly reducing counts with RNA from cells with T-antigen;

thus for the first time there is an opportunity to obtain a meaningful negative. It has been found that an S-positive, T-negative line shows no trace of SV40 mRNA, indicating that the SV40 genome is not present (or functioning) and that the S antigen is likely to be a host antigen whose synthesis is specifically derepressed by SV40.

Significance to Biomedical Research and the Program of the Institute: T-antigens represent a major advance in basic virology and viral oncology, and in potential serodiagnosis of cancer etiology. They offer invaluable tools for studying viral replication, abortive viral infection, and integration of the viral genome into tumor cells.

The Adeno-SV40 hybrids appear to offer a new approach to viral genetics, by allowing propagation of gene segments which must represent massive deletions from the intact genome.

The discovery of a non-defective Ad.-SV40 hybrid particle is a unique finding in the study of animal viruses and should provide significant impetus toward the exploitation of hybrid-virus systems to understand and ultimately control the viral genome.

Proposed Course: Extensive biological and biophysical studies will be carried out to attempt to elucidate the function of that portion of the SV40 genome in the B-89 hybrid. In addition, biophysical studies will be attempted comparing DNA's of each of the clonally stable segments of the Ad2-SV40 hybrid populations to attempt to demonstrate biophysical differences which should provide insight into the markedly different biological properties of these populations.

Attempts to separate non-hybrid particles in the defective Ad-SV40 hybrid populations will be continued to confirm our preliminary finding and to pursue the possibility of preparative separations.

Efforts will be made to develop a very sensitive precipitation test using radioactive tracers to study T-antigen systems.

Honors and Awards:

Invited addresses:

Dr. Rowe - New York University; Jefferson Medical College; Industry Lecture of the Philadelphia Branch of the American Society for Microbiology; University of Toledo; American Association of Immunologists

Dr. Lewis - Duke University Medical Center; American Association of Immunologists

Publications:

Allison, A.C., and Black, P.H.: Lysosomal changes in lytic and non-lytic infections with the Simian Vacuolating Virus (SV40) J. Nat. Cancer Inst., in press.

Black, P.H.: The SV40 T-antigen. Ann. Symp. Tissue Culture Assn., 61-72, 1967.

Black, P.H.: Cancerization *in vitro*. Proc. of the D.C. Chapter of the Soc. Exp. Biol. and Med., in press.

Oxman, M.N., Rowe, W.P., and Black, P.H.: Studies of Adenovirus-SV40 Hybrid Viruses, VI. Differential effects of interferon on SV40 and adenovirus T-antigen formation in cells infected with SV40 virus, adenoviruses, and adenovirus-SV40 hybrid viruses. PNAS 57: 941-948, 1967.

Oxman, M.N., Baron, S., Black, P.H., Takemoto, K.K., Habel, K., and Rowe, W.P.: The effect of interferon on SV40 T-antigen production in SV40-transformed cells. Virology 32: 122-127, 1967

Oxman, M.N.: Some behavioral studies of simian virus 40 (SV40). Archiv fur die ges Virusforschung 22: 171-187, 1967.

Rowe, W.P.: Virus-specific antigens in viral tumors. Cross-Reacting Antigens and Neoantigens. J. Trentin, Ed., Williams and Wilkins Co., Baltimore, 74-84, 1967.

Rowe, W.P., and Lewis, A.M., Jr.: Serologic surveys for viral antibodies in cancer patients. Cancer Research, in press.

Serial Number NCI 4900
1. Viral Carcinogenesis
   Branch, OASDVO, Etiology
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967-June 30, 1968

Title: Spontaneous and Virus-Induced Neoplastic Transformation in vitro (mouse cells and inbred hamster cells).

Previous Serial Number: NCI 4900

Principal Investigator: Charles W. Boone, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.5
Professional:	.5
Other:	1

Project Description

Objectives: (1) To prepare a liquid nitrogen repository of viable-frozen cells collected in large numbers during the process of malignant transformation in vitro. (2) To quantitatively define the specific functional and structural attributes of cells undergoing malignant transformation.

Methods Employed: This program is based on the setting up of a liquid nitrogen repository containing large batches of viable frozen cells collected sequentially from continuous in vitro strains of mouse cells and inbred hamster cells during the process of neoplastic transformation. A series of viable frozen batches of spontaneously transforming mouse cells is nearing completion. A similar series of inbred hamster cells has been started. SV40 virus transformation of the mouse cell sequence has been performed. Adenovirus transformation of the hamster cell sequence is planned.

Major Findings: A total of five mouse cell strains, two of whole embryo, two of embryonic skin, and one of weanling spleen, have been stored at the fifth transfer generation in quantities of 70-80 vials each 5 x 10^6 cells per vial. One mouse embryo skin strain, #238, has been carried through to the 40th transfer generation, from which 50 or more vials, 5 x 10^6 cells per vial, have been stored at the following generations: 5, 10, 13, 15, 18, 20, 23, 25, 28, 30, 33, 35, 38, 40. To date, comprehensive tumorigenicity studies of every fifth transfer generation, using both the intraocular, and intramuscular routes of inoculation in newborn and weanling, radiated and nonirradiated mice, have failed to show spontaneous neoplastic transformation of strain #238. The absence of transformation at such late passage levels is distinctly unusual since the high majority of mouse cell lines do show transformation by this time. The low back of spontaneous transformation in strain #238 will be useful when the line is used to study induction of neoplastic transformation with viruses, hydrocarbons, and radiation. A weanling mouse spleen cell line has been passaged in an identical manner to strain #238, and tumorigenicity studies performed. An anaplastic fibrosarcoma developed within the anterior chamber in one of ten mice inoculated with eight passage cells seven months after inoculation. This spontaneous neoplastic transforming line will serve to compare with cell lines transformed by other means.

Significance to Biomedical Research and the Program of the Institute: The technique of sequentially storing viable-frozen batches of cells from an in vitro strain while it is undergoing neoplastic transformation allows the close comparison of batches from different "transforming series," each induced by a different class of carcinogenic agent (viruses, hydrocarbons, radiation), all of which have been started from the same cell line.

Proposed Course: Three years

Publications:

Boone, C.W., and Dupree, L.T.: Quantitative tumorigenicity assays using the anterior chamber of the mouse eye. Accepted for publication in Cancer Research. Submitted February 21, 1968.

Serial Number NCI-4903
1. Viral Carcinogenesis Branch, OASDVO, Etiology
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967-June 30, 1968

Title: Resolution of Mixtures of Viable Cells from Disaggregated Solid Tumors

Previous Serial Number: None

Principal Investigator: Charles W. Boone, M.D., Ph.D.

Other Investigators: Thomas G. Pretlow, M.D.

Cooperating Units: None

Man Years

Total:	2.0
Professional:	1
Other:	1

Project Description

Objectives: (1) To develop optimal methods for disaggregating solid tumors into suspensions of single, viable cells. (2) To prepare pure tumor cell fractions from these mixed cell suspensions.

Methods Employed: (1) Methods of solid tumor disaggregation by trypsin, EDTA, tetraphenylboron, and mechanical procedures are being evaluated. (2) The principal separation method now in use is zonal centrifugation in density gradients composed of Ficoll dissolved in suspension culture medium. The large capacity A-XII zonal rotor developed by Norman Anderson is being used in addition to density gradients in conventional 100 ml polycarbonate centrifuge tubes.

Major Findings: A method for the isolation of pure cell fractions from mixtures of suspended cells in now well established. Mixtures of cells of two different types, such as rabbit thymocytes, human acute leukemia cells, HeLa cells, and Ehrlich carcinoma cells, can be separated with over 90%

purity. In order to predict the sedimentation behavior of different cells, a book of charts giving the sedimentation position versus time of cells in a density gradient, under standard conditions of gradient composition, angular velocity, and temperature, was prepared with the use of a computer program based on the differential sedimentation equation. Recently, a method for the analytical integration of the sedimentation equation was developed, based on the finding that the viscosity of Ficoll solutions is a simple exponential function of their concentration. This obviates the need for a computer and allows the flexible variation of the five different variables determining the sedimentation position of a cell. In addition, a new principle for separating cells or macromolecules by density gradient centrifugation was developed. This concerned the theoretical development and experimental verification of the fact that the resolution attainable by density gradient centrifugation is markedly increased by moving the entire centrifuge tube as close as possible to the axis of revolution. A special rotor was constructed and is now in use. A number of transplantable mouse tumors are being carried in the laboratory and work is proceeding toward the isolation of pure tumor cell fractions from these tumors. Mouse myelomas, melanomas, mast cell tumors, hepatomas, adrenal carcinomas, breast tumors, and Ehrlich carcinomas are in production.

Significance to Biomedical Research and the Program of the Institute: The goal of isolating pure tumor cell fractions from solid human tumors will have immediate application to the screening of human tumors for virus specific messenger RNA and in research related to virus-induced, tumor-specific, transplantation antigens.

Proposed Course: Three years

Publications:

Boone, C.W., Harell, G., and Bond, H.E.: The resolution of mixtures of viable mammalian cells into homogeneous fractions by zonal centrifugation. J. Cell Bio., 36: 369-378, 1968.

Pretlow, T.G., and Boone, C.W.: Mammalian cell gradient centrifugation: a new rotor. Submitted to Science, March 25, 1968.

Serial Number NCI-4904
1. Viral Carcinogenesis Branch, OASDVO, Etiology
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967-June 30, 1968

Title: Genetic Studies of SV40 Virus Mutants

Previous Serial Number: None

Principal Investigator: George Todaro, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	2.0
Professional:	1
Other:	1

Project Description

Objectives: (1) To prepare SV40 mutants which have lost their ability to transform cells and to produce tumors. (2) To prepare SV40 variants which have high transforming ability. (3) To discover SV40 recombinants resulting from mixed infection with SV40 mutants.

Methods Employed: An FUDR selection method described by Pollack et al will be used to select cell mutants from SV40 tumor cell populations which do not form morphologically transformed colonies and which have reduced or absent ability to produce tumors. Infectious virus will be recovered from these cells by the Sendai fusion method, and tested for transforming capacity in hamster mouse and human cell lines. Ability to induce T-antigen and transplantation antigen, ability to induce cellular DNA synthesis, and ability to produce tumors in newborn hamsters will also be tested.

Major Findings: Preliminary experiments done in collaboration with Dr. T. Takemoto of NIAID indicate that a virus which is recovered by Sendai fusion from a transformed non-virus-containing cell line has a more efficient transforming capability than the original virus. Repeated cycles of transformation and recovery should therefore further select for high transforming variants.

Significance to Biomedical Research and the Program of the Institute: By the means described for obtaining mutants and for producing viral recombination, it is hoped that a detailed genetic analysis of the small DNA tumor virus, SV40, can be started. The significance of this in terms of elucidating the molecular mechanisms of virus transformation is readily apparent.

Serial Number NCI-4905
1. Viral Carcinogenesis Branch, OASDVO, Etiology
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967-June 30, 1968

Title: The Isolation and Characterization of Virus-Induced Tumor-Specific Transplantation Antigens

Previous Serial Number: None

Principal Investigator: Charles W. Boone, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	2.0
Professional:	.5
Other:	1.5

Project Description

Objectives: (1) To develop a method for isolating pure plasma membranes from mammalian cells. (2) To apply the plasma membrane isolation method to virus-transformed tumor cells and homologous normal cells, and to characterize the virus-induced tumor-specific transplantation antigen located on the plasma membrane.

Methods Employed: Subcellular fractionation techniques are used to isolate the plasma membranes from HeLa cells. Each step is monitored by electron microscopy and phase microscopy. The plasma membranes are marked with a I-125 labeled antibody, which permits the plasma membrane fraction to be followed through the various separation procedures. Enzyme markers, in particular membrane specific ATPase, are also used. The isolation of virus transformed cell membranes from cells grown *in vitro* requires very large-scale production of monolayer cultured cells. A large-scale monolayer cytogenerator has been designed and is under construction with the assistance of biomedical engineers from Melpar, Inc. The yield/cost efficiency of this device promises to be superior to any commercially available device at this time.

Major Findings: Work is now completed on a method for the isolation of pure plasma membranes from HeLa cells. A manuscript is in preparation.

Significance to Biomedical Research and the Program of the Institute: The isolation of a purified virus-induced tumor transplantation antigen has both diagnostic and therapeutic significance in relation to human cancer. The purified antigen would permit diagnostic screening of samples of human tumor tissue and also the testing of its efficacy in protecting animals against tumor cell challenge.

Proposed Course: Three years

Serial Number NCI-4906
1. Viral Carcinogenesis Branch, OASDVO, Etiology
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967-June 30, 1968

Title: Elucidation of the Difference Between Normal and "Transformation Prone" Human Cells

Previous Serial Number: None

Principal Investigator: George Todaro, M.D.

Other Investigators: Stuart Aaronson, M.D.

Cooperating Units: None

Man Years

Total:	2.0
Professional:	1
Other:	1

Project Description

Objectives: (1) To develop a rapid assay for cellular sensitivity to viral transformation suitable for screening human populations. (2) To produce adenovirus transformation of human cells, using cells which are highly susceptible to SV40 transformation. (3) Determination of the reason for the difference between normal and susceptible human cell strains with regard to transformation by SV40 virus.

Methods Employed: The customary methodology pertaining to cell culture, use of anti-viral antisera, interferon, T-antigen and V-antigen assay, virus plaque assay, etc., will be used.

Major Findings: A markedly increased susceptibility to transformation has been found with cells from seven individuals with Fanconi's anemia and five individuals with Down's syndrome. Both of these genetic diseases are associated with a high incidence of spontaneous tumors. In addition, cell strains from three out of five members of a "sarcoma prone" family exhibited very high susceptibility to transformation. The quantitative assay which is used to measure susceptibility to transformation determines the fraction of cells which can form transformed clones after exposure to the virus. With normal human diploid cells at a multiplicity of infection of 1,000, this fraction is .02-.05 percent. With the cells from the individuals with Fanconi's anemia, and the sarcoma prone family, the transformation frequency is 10 to 100 times higher. With Down's syndrome it is 3 to 5 times higher.

Significance to Biomedical Research and the Program of the Institute: In relation to the rescue of integrated viral genomes from human cancers: If a large number of cultured human tumors were to be tested for the rescue of an integrated viral genome by co-cultivation with a human indicator cell strain, a strain which is highly susceptible to transformation by adenovirus, and, if possible, other human viruses would be very desirable. The strain showing almost a hundred-fold increase in susceptibility to SV40 virus transformation could be the indicator strain desired.

In relation to the screening of human tumor cells and autologous normal fibroblasts for transformation sensitivity: The presence of an integrated viral genome in a human tumor cell could be reflected by a change in the susceptibility to transformation by another virus. The single fibroblast culture from a human sarcoma patient tested so far showed a marked reduction in susceptibility to transformation compared to normal cells. Reduction in transformation susceptibility might indicate the presence of an occult "interfering" virus. Further testing of more human tumor patients for transformation susceptibility is therefore strongly indicated.

Serial Number NCI-4923
1. Viral Carcinogenesis Branch,
   OASDVO, Etiology
2. Solid Tumor-Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967-June 30, 1968

Title: Identification of Type-Specific and Group-Specific Herpesvirus Antigens

Previous Serial Number: None

Principal Investigator: Berge Hampar, D.D.S.

Other Investigators: Theodore Pincus, M.D.
David A. Stevens, M.D.

Cooperating Units: A. L. Notkins, M.D. (NIDR)
M. G. Mage, D.D.S. (NCI)

Man Years

Total: 3.8
Professional: 2.5
Other: 1.3

Project Description

Objectives: (1) To separate antisera prepared in rabbits against crude herpesvirus antigens into antibody classes which react with specific virion and/or nonvirion associated antigens.
(2) To determine the degree of cross-reactivity by neutralization tests between antibody classes reacting with specific antigens of the known herpesviruses and the human tumor cell-associated herpes-like virus.

Methods Employed: Standard tissue culture and virological methods are used. Viruses are plaque-purified and propagated in primary rabbit kidney (PRK) cells, baby hamster kidney (BHK-21) cells, and human fibroblast (WI-38) cells. Rabbits are used for immunization and preparation of antibodies. Virus neutralization tests are carried out using the plaque reduction technique. The separation of antisera into 7S and 19S components is done chromatographically, using Sephadex G200. Salt precipitated globulins are conjugated with fluorescein isothiocyanate, and fluorescent antibody tests are carried out using both the direct and indirect techniques.

Major Findings: The first phase of these studies involved the characterization of rabbit 7S and 19S antibodies isolated 7 days (early) and 7 weeks (hyperimmune) post-immunization with respect to their ability to neutralize isologous herpes simplex virus (HSV). The parameters studied included the ability of these antibody classes to neutralize virus in the presence or absence of complement (C'), and the ability of these antibodies to sensitize virus for subsequent neutralizatio by anti-γ globulin. The results indicated that early and late rabbit 7S and 19S anti-HSV antibodies possess distinctive neutralizing properties. The early 7S and 19S antibodies showed negligible neutralization titers in the absence of C'. When C' was added, however, these antibodies showed significant neutralization titers. The early 7S antibody differed from the early 19S antibody in that only the former showed significant neutralization titers with anti-γ globulin. The neutralizing activities of the early 7S and 19S antibodies in the presence of C' were reduced significantly following treatment of the antibodies with 2-mercaptoethanol (2-ME). Treatment with 2-ME, however, did not effect the ability of the early 7S antibody to sensitize virus for subsequent neutralization by anti-γ globulin.

The neutralization titers of the late 7S antibody were not enhanced significantly in the presence of C'. This differed from the C'-requirement shown by the early 7S antibody. However, the neutralization rate constants (K values) of the late 7S antibody were enhanced in the presence of C', and this enhancement was sensitive to inactivation by 2-ME. The late 19S antibody showed low neutralization titers in the absence of C', but its neutralization titers were enhanced significantly in the presence of C'. Treatment of the late 19S antibody with 2-ME reduced its neutralization titers to low levels, even when C' was added to the reaction mixture. Both the late 7S and 19S antibodies could sensitize virus for subsequent neutralization by anti-γ globulin. This property of the late 19S antibody was not observed with the early 19S antibody. The ability of the late 19S antibody, but not the late 7S antibody, to sensitize virus for subsequent neutralization by anti-γ globulin was sensitive to inactivation by 2-ME.

Many of the properties noted with early and late 7S and 19S anti-HSV rabbit antibodies were also noted with anti-SA8 antibodies. It is probable that these properties are characteristic for many, if not all, herpesvirus antibodies made in rabbits.

Studies were also carried out to determine whether rabbit 7S and 19S anti-HSV antibodies differed in their ability to differentiate between antigenic variants of HSV. For these studies, two intratypic variants of HSV were employed which could not be differentiated by reciprocal neutralization tests (neutralization titers), but which could be differentiated by reciprocal neutralization kinetic tests. The early 19S antibody, but not the early 7S antibody, could differentiate between these HSV variants in the presence of C'. The late 19S antibody could differentiate between these variants in the presence or absence of C'. The late 7S antibody could differentiate between these HSV variants in the absence, but not in the presence, of C'. Further, the ability of the late 7S antibody to differentiate between these HSV variants was destroyed following treatment of the antibody with 2-ME. These results suggest (1) that the specificity of the 7S and 19S antibodies differ, and (2) that the 2-ME sensitive portion of the late 7S antibody population was responsible for both the enhanced K values noted in the presence of C', and for the ability of this antibody to differentiate between intratypic HSV antigenic variants.

Studies similar to those described above have also been carried out between HSV and SA8 virus which show reciprocal cross-reactivity. These studies were carried out by determining antibody neutralization titers. The early 7S and 19S antibodies showed reciprocal cross-reactivity in the presence of C'. The late 19S antibody, however, showed no reciprocal cross-reactivity in the presence or absence of C'. The late 7S antibody showed some cross-reactivity in the absence of C', but the degree of cross-reactivity was enhanced significantly in the presence of C'. Further, the enhancement by C' of the cross reactivity by the late 7S antibody was sensitive to inactivation by 2-ME.

Significance to Biomedical Research and the Program of the Institute: The above studies show that early and late rabbit 7S and 19S antibodies to herpesviruses differ (1) in their ability to neutralize isologous virus under various conditions; (2) in their ability to differentiate between intratypic virus antigenic variants; and (3) in their ability to cross-react with heterologous herpesviruses. These findings, in themselves, are of interest, both in terms of defining the properties of different antibody classes and in characterizing the cross-reactions between herpesviruses. These studies seem of particular importance to the work presently being carried out to determine the possible relationship between herpesviruses and human cancer. One of the major limitations in the work to date has been the inability to isolate an infectious agent from tumor cells showing evidence of harboring herpes-like viruses. Further, the immunologic studies carried out in an attempt to identify these virus-like particles have employed human sera, which contain antibodies to many viruses. It seems clear that positive identification of these virus-like particles, in the absence of an infectious agent, requires the solution of two problems. First, a means must be found to determine whether a positive reaction of human sera against these herpes-like particles is due to antibody directed against the particle in question, or whether the activity of the antibody is directed against a cross-reacting virus being harbored in the same host. In this respect, the studies being carried out in this laboratory are relevant, since they indicate that specific antibody activity is associated with certain classes of antibody. Preliminary evidence suggests that human antibodies share many properties in common with those found for rabbit antibodies. Second, the herpes-like particles in tumor cells may represent incomplete or defective particles. Consequently, attempts to identify these particles by using antisera prepared in animals against infectious (complete) virus particles may preclude reactivity. It seems that the positive identification of these virus-like particles or the rejection of these particles as being one of the known herpesviruses would require testing with antibodies which react with different components of the virion or antibodies which react with nonvirion associated antigens. The studies being carried out in this laboratory, though preliminary, suggest that different classes of rabbit antibodies may be reacting with different virion and/or nonvirion associated antigens.

Proposed Course: Studies will be continued to further delineate the properties of 7S and 19S antibodies isolated from rabbit and human sera with respect to their specificity for isologous and/or heterologous herpesvirus associated antigens.

Publications:

Hampar, B., Notkins, A.L., Mage, M.G., and Keehn, M.A.: Heterogeneity in the properties of rabbit neutralizing antibodies against herpes simplex virus. J. Immunol. 100: 586, 1968.

Hampar, B., Mage, M.G., and Keehn, M.A.: Heterogeneity in the ability of rabbit 7S and 19S antibodies to differentiate between intratypic antigenic variants of herpes simplex virus. J. Immunol. (in press).

Stevens, D.A., Pincus, T., Burroughs, M.A.K., and Hampar, B.: Heterogeneity in the degree of cross-reactivity shown by rabbit 7S and 19S antibodies against herpes simplex virus and a simian herpesvirus (SA8) (in preparation).

Summary Report
Viral Biology Branch
July 1, 1967 to June 30, 1968

Office of the Chief:

Dr. Valentine, presently in the Office of the Chief, has collaborated in an ultrastructural and biophysical study of mammalian and avian oncogenic viruses. Both murine sarcoma virus (MSV) and Rous sarcoma virus (RSV) transformed rat and hamster cells. Transformed rat cells produce virus particles which are incapable of inducing sarcoma. However, when leukemia virus is added, the particles produced become infectious for rat embryo cells. This is strikingly similar to the "non-producer" situation in the RSV chick embryo system. Other studies on the nucleocapsid of RSV have revealed the presence of particulates with a diameter of approximately 60 mμ after partial disintegration of the RSV particles.

Projects are also being developed which will attempt to demonstrate what happens to viral nucleic acid in the eclipse phase after viral infection and before the production of new virions. Six articles have been written in the Office of the Chief during this fiscal year. Two have been published; 4 others are in press. The Chief as co-editor of the series on Ultrastructure in Biological systems was responsible for the appearance of a new monograph on Ultrastructure of the Kidney. Two other monographs, one on the Nucleus, the other on biological Membranes, are in press.

It should be noted that at least 80% of the time of the Chief of the Viral Biology Branch is taken up with: 1. The SVLP, 2. reviews of applications for duty-free entry of foreign scientific equipment (The Florence Agreement Committee), 24 applications a month. 3. reviews of grant applications as a member of the Molecular Biology Study Section, and 4. Administration of the Viral Biology Branch.

Electron Microscopy Section

(1) By the use of a dual spinner flask in which the two sections were separated by a 220 mμ Millipore filter the herpes-type virus associated with Burkitt lymphoma has been demonstrated to infect other human cell lines. The transfer of virus and infectivity was demonstrated by electron microscopy. Three cell lines from a leukemic monkey have been established and have been demonstrated to carry a herpes-type virus. Inoculation of cell packs and cell-free fractions from these lines into newborn Rhseus monkeys has been carried out. (2) In another study an evaluation of the ultrastructural changes induced by prednisolone pretreatment of mice infected with Moloney

leukemia virus is being carried out. (3) Using the technique of ultrastructural cytochemistry it has been demonstrated that the RNA in both intracytoplasmic A particles and B particles is removable by trypsin because trypsin cleaves peptic bonds of the nucleic acid. (4) The ultrastructure of "cell hypertrophy" tumors has been investigated. These tumors of fish are caused by a microsporidian. This is a very simple organism which becomes morphologically and physiologically incorporated into the host cell. This relationship is analogous to that of certain viruses. (5) An ultrastructural study of advancing and regressing virus-induced sarcomas has demonstrated that virus is most readily demonstrated at the height of the advancing tumor. Virus could not be demonstrated in regressing tumors. (6) Electron microscopic analysis of tissue culture isolates from biopsies of human malignancies has demonstrated the presence of both a herpes-type virus and a myxovirus type. (7) Of 26 plasma pellets from patients with various malignancies, 3 were found to be positive for C type particles. This work is being phased out with a shift in emphasis to the study of lymph nodes from patients with leukemia, lymphoma and Hodgkin's disease.

Technical studies:

A second generation (more sophisticated) image intensifier is being adapted for use with one of the Section's electron microscopes.

A new high intensity grid cap has been developed which greatly increases beam intensity. Five articles have been written by members of this Section during this fiscal year. The papers are either published or are in press.

Microbiology Section:

An attempt is being made to determine a one-step growth cycle for Moloney sarcoma virus (MSV). In order to do this it will be necessary to obtain a high titer, non-toxic MSV preparation. MSV has been replicated in vitro and the virus concentrated by Aquacide treatment. This material has a ten-fold higher titer and is non-toxic.

In another study it has been demonstrated in vitro that interferon under certain conditions prevents infection by MSV.

Two methods for assaying leukemia virus were tested and found to be satisfactory. One method involved the ability of leukemia-infected cells to prevent MSV focus formation, the other on the ability of supernates from these cultures to increase MSV focus by "incompetent" virus. These methods reduced the assay time from 6 months to 3 weeks.

In a study of chemotherapy in tissue culture, it has been shown that human leukocytes in long-term culture will serve as a useful tool for the study of drug-target cell interactions. The success of these preliminary studies means that with further effort it should be possible to use the human leukocyte culture model to design more efficient anti-leukemic drug regimens.

Sero-epidemiological studies have been carried out on a series of human cell lines. Herpes-type virus (HTV) has been found in all infectious mononucleosis lines. Fifty percent of the sera from normal persons contained anti-HTV anti-body while 100% of the sera from Burkitt and infectious mononucleosis patients contained it.

Massive inocula of cells and tissue culture supernates have been made into both monkeys and chimpanzees. To date no abnormalities have been induced.

Two papers are in press from this Section.

Virus Studies Section:

Two liposarcoma biopsies which had been frozen at -190°C for several years were grown out in tissue culture. Both of these lines were found to contain an SV-5 like myxovirus by electron microscopy and by immunological methods. The results of the unimmunological tests suggest that rubella may also be present.

Three articles have been written in this Section during this fiscal year. One is published; the other 2 are in press.

Serial No. NCI-4872
1. Viral Biology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on viral replication.

Previous Serial Number: None.

Principal Investigators: Albert J. Dalton and Artrice F. Valentine

Other Investigators: None.

Collaborating Units:

Dr. H. B. Andervont
Dr. John Bader
Dr. Y. Hirshaut
Dr. M. Potter
Dr. W. P. Rowe, NIAID
Dr. L. Dmochowski, M. D. Anderson Hospital and Tumor Institute

Man Years:

Total:	5.00
Professional:	2.00
Other:	3.00

Project Description

1. Animal studies.

(a) Dr. Dalton in collaboration with Dr. G. Smith and Dr. Rowe is continuing studies on the visualization of the lymphocytic-choriomeningitis virus. The particles varying in size from 50 to 300 mμ mentioned in last year's report have been found only in green monkey kidney tissue cultures infected with LCM and not in control cultures. However, the particles have not been found in human embryo kidney tissue cultures infected with LCM. The significance of this is not clear at present. The particles contain one or several ribosome-like bodies. These bodies have been shown to be removed by ribonuclease.

(b) Dr. Dalton in collaboration with Doctors Andervont and Potter has completed a study on the intracytoplasmic A type particles associated with the mammary tumor agent. Plasma cell tumors were induced in BALB/c An mice which had been foster nursed on C3H mice to introduce the mammary tumor agent. Cells of both primary and 1st generation transplants of these plasma cell tumors contained intracytoplasmic type A particles. These particles have not been observed in plasma cell tumors of BALB/c An mice previously. This experiment demonstrates that these particles are transmissible through milk and further relates them to the mammary tumor agent.

(c) In collaboration with Dr. John Bader, Dr. Valentine undertook a study of ultrastructural and biophysical characteristics of mammalian and avian cells infected with RNA-containing tumor viruses. The first part of this project involved an examination of cultured mammalian cells transformed with Rous sarcoma virus (RSV) and murine sarcoma virus (MSV) by means of electron microscopy and radioactive labelling techniques. Both rat cells and hamster cells transformed with either RSV or MSV were investigated. There were no particles observed in the hamster cell lines transformed with the RSV or MSV which could be detected with either electron microscopy or radioactive labelling techniques. However, electron microscopy revealed virus particles in the rat cells transformed with either the MSV or RSV. Radioactive labelling techniques showed these particles to have a buoyant density characteristic of RNA-containing tumorigenic viruses, i.e., 1.16 g/ml in sucrose gradient and a high molecular weight RNA which sedimented in the region of 65-70 S. These virus particles were found to be non-infectious when tested on rat embryo, chick embryo or baby hamster kidney cells. However, when murine leukemia virus (either Rauscher or Moloney strain) was added to the rat cells transformed with MSV, virus particles which were infectious for rat embryo cells were produced. This supported the hypothesis that the rat cells transformed with MSV (Obtained from Dr.R. Ting and originally designated R-MSB$_1$) were analogous to the non-producer cells of the Rous sarcoma virus, Bryan strain,-chick embryo cell system. That is, the cells containing the MSV genome produced virus particles which were unable to re-infect rat embryo cells. When the rat cells transformed with MSV were tested against group specific antigens of murine leukemia virus, the complement-fixation test was negative as opposed to a positive CF result with the Rauscher leukemia virus (JLSV$_5$) used as a control in these studies.

Because of the apparent similarity of the MSV system to Rous sarcoma virus as exemplified by the rat cells transformed by MSV, investigations are now in progress to isolate clonal stocks of rat embryo cells transformed with murine sarcoma virus. As the clones are isolated, they will be checked by electron microscopy for the possible production of leukemia virus. Also, the cells of the clones will be examined for significant ultrastructural characteristics.

In collaboration with Dr. John Bader, Dr. Valentine is currently involved in studies on the analysis of the nucleocapsid of avian tumor viruses. The nucleocapsid of Rous sarcoma virus has been examined by both thin sectioning and negative staining techniques. The virus particles are first treated with a detergent which removes the outer envelope and the treated virus preparations are then banded in density gradients. The nucleocapsids (cores) are found to have a buoyant density of 1.26g/ml. When these bands are checked by electron microscopy, particulate structures are observed in both thin sections and grid preparations negatively stained with either potassium phosphotungstate or potassium silicotungstate. These particles have an average diameter of 60 mµ in negatively stained preparations and 55 mµ in thin sections.

Dr. Valentine, in collaboration with Dr. John Bader is at present studying the development of autoradiography techniques for electron microscopy. Preliminary studies are in progress for the application of autoradiography techniques for the study of cultured cells infected with RNA-containing tumor viruses. Cells growing in growth medium were transferred to medium containing ^{3}H-labelled uridine and the uptake of the radioactive labelled nucleoside into the cell will be observed. These experiments are designed for the learning and development of the technique of electron microscopy autoradiography. Different methods of fixation and processing of the samples are being tried. When satisfactory results are obtained in this laboratory, using the test system described here, the autoradiooraphy will be employed in the study of the adsorption, penetration and replication of Rous sarcoma virus and other RNA-containing tumor viruses.

Publications:

Bader, J. P. and Valentine, A. F.: Biochemical studies on RNA-containing tumor viruses. Symposium on Comparative Leukemia Research, Paris, France, 1967. Accepted for publication.

Dalton, A. J. and Manaker, R. A.: The comparison of virus particles associated with Burkitt lymphoma with other herpes-like viruses. In Mandell, M. (Ed.): Carcinogenesis; A Broad Critique. Houston, The Williams & Wilkins Co., 1967, pp. 59-90.

Publications (Cont.)

Dalton, A. J. and Zeve, V. H.: A review of studies with the electron microscope on human leukemia and Burkitt's tumor. Cancer Res. 27: 2465-2470, 1967.

Dalton, A. J., Rowe, W. P., Mitchell, E. Z. and Pugh, W. E.: Detection of virus particles in leukemia-lymphoma by electron microscopy. Accepted for publication. Proc. International Conf. on Leukemia-Lymphoma, 1967.

Dalton, A. J. and Haguenau, F. (Eds.): Ultrastructure of the Kidney. New York, Academic Press, 1967, 240 pp.

Valentine, A. F. and Bader, J. P.: Production of virus by mammalian cells transformed with Rous sarcoma and murine sarcoma viruses. Accepted for publication. J. Virology.

Serial No. NCI-4870
1. Viral Biology Branch
2. Electron Microscopy Sect.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Ultrastructural studies on normal and malignant cells and related viruses.

Previous Serial Number: NCI-4870

Principal Investigator: Victor H. Zeve

Other Investigators:

Dr. Herbert T. Abelson
Elisabeth Z. Mitchell
Bolivar J. Lloyd, Jr.
Dr. Gilbert H. Smith
Dr. Sanford H. Vernick

Collaborating Units:

Dr. H. B. Andervont
Dr. P. B. Blair, University of California
Mr. George Christov, RCA
Dr. Harold Hoffman
Dr. Jack Jones, University of Maryland
Dr. John Landon, Bionetics Research Laboratory
Dr. Kalman Perk
Dr. Victor Sprague
Dr. Sarah E. Stewart

Man Years:

Total:	11.0
Professional:	6.0
Other:	5.0

(1) Dr. Victor H. Zeve has developed a technique for infecting virus negative human cell lines with the herpes agent associated with Burkitt tumor and human leukemia. The method involves the cell-free transfer of virus across a 220 mμ Millipore filter in a dual spinner flask. Dr. Zeve and Mrs. Joan Kondratick have established cell lines from a leukemic monkey (L-18; Brooks AFB, San Antonio, Texas),and the spleen, bone marrow and kidney cultures contain the herpes type virus. Inoculation of newborn Rhesus monkeys with cell packs and cell free material have been initiated and will continue. In collaboration with Dr. Jack Jones, Department of Entomology, University of Maryland, Dr. Zeve studied the fine structure of the gastric caecae in *Aedes egypti* larvae. The resulting manuscript was submitted to the *Journal of Cell Biology* for publication.

In collaboration with Dr. John Landon, Bionetics Research Laboratory, electron microscopic studies of tissue culture cell lines isolated from the buffy coats of chimpanzees and baboons with the herpes type virus occurring naturally are being continued.

Dr. Zeve is presently designing an adapter mechanism for the RCA microscope which will incorporate an image intensifier. Adaptation of a new S. E.C. orthicon camera with T.V. readout will enable investigators to view materials at reduced beam intensity and greatly reduced high voltage acceleration. Images can be stored on magnetic tape or stored electronically on computer disc recorders for future comparison.

(2) Dr. Herbert Abelson continued studies initiated at the University of Colorado Medical Center on Nephrogenic Diabetes Insipidus and were completed with the first fine structure description of this disease. At Microbiological Associates in Walkersville, Md., a study is in progress to evaluate the effects of prednisolone pretreatment of mice infected with Moloney leukemia virus. In collaboration with Dr. Harold Hoffman, viral antibodies are being labeled with horseradish peroxidase in an attempt to localize viral antigens in tissue culture and within the glomerular basement membrane of animals with autoimmune nephritis.

(3) Dr. Gilbert H. Smith has completed studies on the ultrastructural cytochemistry of the mammary tumor virus. Enzyme digestion studies have been continued to classify the role of trypsin in the demonstration of the RNA associated with the intracytoplasmic A particle and nucleoid of the extracellular B particle. The results have indicated that trypsin is necessary for the cleavage of peptide bonds in the basic patterns which apparently are closely associated with the ribose nucleic acid in each type of particle.

In collaboration with Dr. P. B. Blair at the University of California comparative studies on the mammary tumor virus (MTV) and nodule-inducing virus (N.I.V.) in C3H/Stw mice are in progress.

Experiments in collaboration with Dr. H. B. Andervont to test for the presence of N.I.V. in MTV-free RIII mice is nearing completion.

(4) Dr. Sanford H. Vernick in collaboration with Dr. Kalman Perk and Mr. Bolivar J. Lloyd, Jr. has been studying the erythrocyte host response in Moloney murine leukemia.

The red blood cells of 2-day old normal and 8-week old Moloney leukemia-infected BALB/c mice were examined using frageligraphy, electron microscopy, and hemaglobin electrophoresis. Results of frageligraphy revealed similar bimodal curves representing two RBC populations for the young normal and adult leukemic animals. Electron microscopy confirmed these two populations and revealed similarities in morphology between RBC's from 2 day old normals and 8-week old leukemic animals. Electrophoresis showed no evidence of fetal hemaglobin in any of the samples. Thus it is assumed the cells from the osmatically more resistant second populations in the leukemic animals are not fetal type cells in the sense of hemaglobin types, but similar to the fetal erythrocytes which start to develop in the fetal liver (12th to 17th day of gestation) and subsequently in the fetal bone marrow (after day 16).

Dr. Sanford H. Vernick and Mr. Bolivar J. Lloyd, Jr. have been collaborating with Dr. Victor Sprague in studying an experimentally induced tumor in a crab *Callinectes sapidus*.

The tumor is caused by a microsporidian belonging to the genus *Nosema*. Under electron microscopy only diplokarya and stages in sporogony were seen. The fine structure was basically similar to that reported for other microsporidiabut there were some peculiar features. Chief of these were a new kind of polaroplast suspended in a cavity and bristles eminating from the surface of the spore. This genus of parasite is the same as that associated with certain diseases of man called encephalitozooan.

Dr. Sanford H. Vernick in collaboration with Dr. Victor Sprague of the Chesapeake Biological Laboratory has been studying cell hypertrophy tumors.

This type of tumor is found in a fish inhabiting the inshore waters near Solomons, Maryland. The tumor is caused by a microsporidian tentatively identified as *Glugea anomala*. Its fine structure corresponds with the basic pattern revealed by other authors with similar species. A concept of spore morphogenesis in which the polar filament primordium is one-half of the nuclear isthmus present during division of the sporont, is elaborated and its implications discussed. The membrane systems of the parasite and the host cell components appear to be continuous with one another, this being an indication that the membranes are all furnished by the host cell. Lacking mitochondria and a Golgi apparatus the parasite is then considered apart from the membrane system which is common to it and the host cell, a very simple organism consisting of little else besides the genome. This simplicity, its very high degree of morphological and physiological integration with the host cell, and the transformative development of the host cell all suggest an analogy with certain viruses.

Dr. Sanford H. Vernick, Mr. Bolivar J. Lloyd, Jr. and Mr. Harvie Sims have been studying the relationships of the Moloney sarcoma virus to advancing and regressing tumors. Two-week old C57Bl mice were injected with MSV and samples of the growing and subsequently regressing tumor were examined under the electron microscope for presence of virus. At the same time samples of surrounding muscle, spleen, thymus, and lymph node were also taken. Virus was not found in the early advancing tumor or in the regressing tumor but only at the height of advancement of the tumor. This was confirmed by in vitro studies done concurrently by Dr. Kirby Smith. The morphology of the virus was the same as described by other authors. As the tumor regressed the cells once again took on the appearance of normal muscle, the regeneration of the fibers being evident.

(5)Bolivar J. Lloyd in collaboration with Dr. Sarah Stewart has been studying the ultrastructure of virus infected cells in tissue culture cell lines derived from humans and hamsters. Results of these studies were published in the Journal of the N.C.I.

In collaboration with Mr. George Christov, RCA, Mr. Lloyd has been testing and advising in the development of a new high intensity grid cap for the RCA microscope. Preliminary results indicate that the image is greatly enhanced and that sufficient light is now obtainable for high quality micrographs above 15,000 X magnifications. This development represents an outstanding advance in that it greatly enhances the usefulness of the RCA instrument.

Mr. Lloyd continues to investigate the vertical shadowing methods. This utilizes heavy metal deposition on biological materials and permits the visualization of ultrastructural components not seen by other methods.

(6)Mrs. Elisabeth Z. Mitchell in collaboration with Dr. Sarah Stewart has examined pellets of human biopsy materials grown in tissue culture for varying periods of time and passaged via cell free concentrates onto other cell lines. The source materials were lymph node from a patient with Hodgkin's disease and sarcoma tissues from two patients.

The early growth of the Hodgkin's tissue in shaker culture revealed a herpes-type virus. After many passages of the above tissue in monolayer cultures, E.M. examination revealed particles of the myxovirus group in all samples and no herpes particles.

Plasma Pellet Samples from Plasmaphoresis and Dr. Moloney's Lab Received from April 1, 1967 - March 31, 1968

These tissues were examined by Elisabeth Z. Mitchell

Diagnosis	All samples	Different Patients	Total Positives	Total Different Positive Patients	Total + -	Total Different Patients
CML	39	15	3	2	7	5
CLL	10	4				
ALL	2	1				
Malignant mesodermal tumor	4	1				
Infectious mononucleosis	1	1				
Malignant lymphoma	4	1				
Clediak-Higashi syndrome	7	1				
Lymphosarcoma	1	1				
Lymphoproliferative disorder	4	1				
Total patient samples	72	26	3	2	7	5
Normals	6	6				
Total-all samples	78	32	3	2	7	5
Moloney samples						
CML	1	1				

In addition, two lymph node specimens from patients with leukemia from M. D. Anderson Hospital in Texas were examined.

(7) Dr. Gilbert H. Smith has completed studies on the ultrastructural cytochemistry of the mammary tumor virus. Enzyme digestion studies have been continued to classify the role of trypsin in the demonstration of the RNA associated with the intracytoplasmic A particle and nucleoid of the extracellular B particle. The results have indicated that Trypsin is necessary for the cleavage of peptide bonds in basic proteins which apparently are closely associated with the ribose nucleic acid in each type of particle.

In collaboration with Dr. P. B. Blair at the University of California comparative studies on the mammary tumor virus (MTV) and nodule-inducing virus (NIV) in C3H/Stwi mice are in progress.

Experiments in collaboration with Dr. H. B. Andervont to test for the presence of NIV in MTV-free RIII mice is nearing completion.

Publications:

Landon, J. C., Ellis, L. B., Zeve, V. H., and Fabrizio, P. A.: Herpes-type virus in cultured leukocytes from chimpanzees. J. Nat. Cancer Inst. 40: 181-192, 1968.

Smith, G. H.: Cytochemical studies on the mouse mammary tumor virus. Cancer Res. 27: 2179-2196, 1967.

Serial No. 4871
1. Viral Biology Branch
2. Microbiology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Investigations on Viral Oncogenesis

Previous Serial Number: Same

Principal Investigator: Robert A. Manaker, Ph.D.

Other Investigators: Gary Armstrong

Cooperating Units: Dr. Frank J. Rauscher, ASDVO, Etiology, NCI
Dr. John Landon, Bionetics Research Laboratories,
Contract No. PH43-67-661

Man Years:

Total:	8.6
Professional:	1.0*
Others:	7.6

*Note: Does not include 0.7 man years of professional time devoted to activities not directly concerned with personal participation and/or guidance of the laboratory unit. The latter time was spent predominantly on duties as: (a) chairman of the Developmental Research Segment of the Special Virus Leukemia Program; (b) project officer on two large NCI contracts.

Project Description

Objectives:

To isolate, propagate and study known or suspected oncogenic viruses in cultured cells and animals and to conduct collaborative investigations on the role of viruses in the etiology of some spontaneous neoplasms of man.

Methods Employed:

Viruses of the herpes type unrelated to known members of the herpes virus group have been repeatedly detected by electron microscopy in lymphoid cells cultured from peripheral blood or hematopoietic tissues of patients with leukemia or lymphoma. A cooperative study in progress for 2 years was initiated between Dr. Frank J. Rauscher, Jr., Dr. Paul Kotin and this laboratory to determine the response of lower primates to the herpes type viruses administered with and without selected chemical carcinogens. In addition, different routes of inoculation were used. The work was conducted with cell and virus material from Charles Pfizer and Company, Inc., produced under contract and animals were housed at Bionetics Research Laboratories.

The Rauscher strain of murine leukemia virus loses oncogenicity on continued propagation of chronically infected, virus-releasing cultured mouse cells. Such non-leukemogenic virus inoculated into mice protects them on subsequent challenge with leukemogenic Rauscher virus. The ability of the virus to interfere with the development of plaques by mouse sarcoma virus in a selected mouse cell line was used to titrate the leukemia virus indirectly. Other methods are under development. Virus was selected from cultures infected with high dilutions of inoculum to select purified strains. Since leukemia group viruses are ubiquitous in their distribution in all strains of mice, existing virus pools must consist of mixed populations. If purified strains can be obtained, the factors involved in the loss or gain of leukemogenic properties by this virus and the means by which non-leukemogenic virus exerts its protective action may be studied.

Major Findings:

In the cooperative project between Dr. Frank J. Rauscher, Dr. Paul Kotin and this laboratory, herpes-type virus contained in, and extracted from, cultured cells derived from American leukemia and lymphoma patients and from African Burkitt's lymphoma patients was inoculated into lower primates. Some animals also received chemical carcinogens together with virus. The oldest animals have been under observation for about 2 years. Survivors include 126 rhesus, 17 cynomolgus, 4 bonnet, and 1 African green monkey. No gross abnormalities have developed.

Massive inocula of cells containing the herpes type virus were injected into 5 chimpanzees. No significant response has been detected. Intrapulpar or intramandibular administration of this virus in 15 rhesus and 1 cynomolgus monkey has also evoked no abnormalities.

Several passages of poorly leukemogenic Rauscher mouse leukemia virus have been made. Considerable time is required because virus at minimal doses grows slowly in culture. The plaque reduction method has proven satisfactory for detection of leukemia virus infected cultures, but simpler methods are under development.

Significance:

The lower primates provide the best available host for study of viruses suspected of oncogenicity in man. The induction of neoplasms in these animals by a virus consistently found in humans with neoplastic disease would be highly suggestive of comparable activity in man. The relatively short time of observation of the animals on test is insufficient to reach conclusions concerning the significance of the negative response in experimental animals to date.

The leukemia viruses of mice, ubiquitous in their distribution in mouse strains, provide the best available system for study of virus-host relationships.

Proposed Course:

Observations on lower primates infected with human leukemia materials and associated viruses will be continued.

Studies with murine tumor viruses will be expanded.

Serial No. 4874a
1. Viral Biology Branch
2. Microbiology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Investigations on the Etiology of Human Leukemia and Lymphoma

Previous Serial Number: Same

Principal Investigator: Dr. Yashar Hirshaut

Other Investigators: Elizabeth Blackham
Thomas Pry

Cooperating Units: Dr. Seymour Perry, Associate Scientific Director for Clinical Trials, NCI
Dr. Lawrence Chessin, Laboratory of Clinical Investigations, NIAID
Dr. A. J. Dalton, Chief, Viral Biology Branch, NCI
Dr. R. A. Manaker, Head, Microbiology Section, NCI

Man Years:

Total:	1.5
Professional:	1.5
Other:	0

Project Description

Objectives:

1. To study the role of Burkitt-associated herpes-like virus (HLV) in the pathogenesis of lymphoproliferative disorders.

2. To determine the distribution of anti-HLV antibody among normal persons and among patients with leukemia, lymphoma, Hodgkins disease, infectious mononucleosis and lupus erythematosis.

3. To isolate, concentrate and transmit HLV in an effort to discover its pathogenic potential.

Methods Employed:

HLV was detected by direct and indirect immunofluorescent techniques and electron microscopy. Indirect fluorescent antibody methods were also used to identify and titer sera containing anti-HLV antibody.

Virus was isolated and concentrated by alternate filtration and ultracentrifugation from supernatant fluids of HRI-K (P3J) lymphoid cell cultures. Transmission attempts were then made by coincubating the HLV concentrate with a pellet of cultured human lymphoid cells.

Major Findings:

Following a report that HLV was the probable etiologic agent of infectious mononucleosis, we searched 16 long-term leukocyte cultures, derived from nine patients with infectious mononucleosis for the presence of HLV. At least one cell line from each of the nine donor patients was found to harbor HLV.

Fifty per cent of sera from normal persons contained anti-HLV antibody. The incidence in patients with infectious mononucleosis and Burkitt tumor was 100%. About 30% of patients with infectious mononucleosis demonstrated a fall in anti-HLV titers during convalescence, while 10% first developed antibody as their symptoms cleared.

A 1:2000 HLV concentrate was used to infect a previously virus-free lymphoid cell culture, R.P.M.I. 6410. Virus transmission was demonstrated principally by fluorescent antibody techniques. Within 24 hours after coincubation of virus with target cells, many giant cells were seen, the cell count began to fall and there was a marked decrease in viability. Most of the cells which had been exposed to virus were destroyed within four days.

Significance:

Herpes-like viruses (HLV) have been demonstrated in long term leukocyte cultures derived from normal persons and patients with Burkitt tumor, leukemia and lymphoma. The work undertaken represents an effort to determine whether HLV is a pathogenic organism responsible for one or more of the lymphoproliferative disorders.

Proposed Course:

Seroepidemiological surveys will be continued in an attempt to delineate more completely the distribution of anti-HLV antibody among patients with lymphoproliferative disorders and normal individuals. For this purpose the indirect immunofluorescent assay will continue to be used and an attempt will be made to develop other quantitative techniques to corroborate our findings. By testing a large number of additional sera from patients with infectious mononucleosis we hope to gain added insight into any unique relationship that might exist between HLV and this disease.

Further efforts will be made to isolate, concentrate and purify HLV. Virus obtained in this way will then be employed for both in vitro and in vivo infectivity studies. In relation to this, an attempt will be made to develop new methods for quantitating infective virus particles.

Publications:

1. Hirshaut, Y., Manaker, R., Glade, P. R., Chessin, L. N., and Kasel, J. A.: Formal Comment: On the Role of Burkitt HL Virus in the Etiology of Infectious Mononucleosis. Perspectives in Virology, New York, 1968 (in press).

2. Chessin, L.N., Glade, P.R., Kasel, J.A., Moses, H.L., Hirshaut, Y., and Herberman, R.B.: The Circulating Lymphocyte -- Its Role in Infectious Mononucleosis. Annals of Internal Medicine, 1968 (in press).

Serial No. 4874b
1. Viral Biology Branch
2. Microbiology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Investigations on the Control of Human Leukemia and Lymphoma

Previous Serial Number: Same

Principal Investigator: Dr. Yashar Hirshaut

Other Investigators: Elizabeth Blackham
Thomas Pry

Cooperating Units: Dr. Seymour Perry, Associate Scientific Director for Clinical Trials, NCI
Dr. A. J. Dalton, Chief, Viral Biology Branch, NCI
Dr. R. A. Manaker, Head, Microbiology Section, NCI

Man Years:

Total:	1.5
Professional:	1.5
Other:	0

Project Description

Objectives:

1. To bioassay serum concentrations and serum half-lives of anti-leukemic drugs while they are being used to treat patients with acute leukemia.

2. To determine how *in vitro* white cell destruction is related to anti-leukemic drug concentration in the culture medium and length of cell exposure to drug.

3. To use cultured human leukocytes as a model system to predict how cytotoxic drugs may best be used in the treatment of leukemia.

Methods Employed:

For bioassay, aliquot cultures of human leukocytes were exposed in parallel to known quantities of the drug under study and to unknown quantities of drug present in sera taken from patients recently infused with the chemotherapeutic agent. By comparing cell kill achieved with known drug quantities to cell kill achieved by the test sera, it was possible to estimate the drug concentration in the circulation at the time the serum sample was taken from the patient. This assay also made it possible to observe the fall in anti-leukemia drug level with time so that the half-life of the drug could be determined.

To study drug action in vitro, serial dilutions of drug were added to aliquot long-term cultures of human leukocytes and washed off at fixed intervals or left in place for seven days. All cultures were counted on the first and last days of the experiment to compare the growth of treated cultures to growth of controls.

Major Findings:

Bioassay has made it possible for the first time to measure circulating drug levels for vincristine. This drug has a maximum in vivo concentration of approximately 0.4 μg/ml following a total dose of 2 mg administered intravenously. Its half life is about 75 minutes. Concentrations of 6-mercaptopurine in serum have been previously studied by radioassay but only in a few patients. This drug's serum level shortly after infusion of 500 mg/M^2 is about 50 μg/ml. Its half-life is in the vicinity of 60 minutes.

In vitro drug studies have extended our previous observations of the efforts of 6-mercaptopurine, methotrexate, vincristine, and prednisolone on human leukocytes in long term culture. By applying these agents to additional cell lines it has been possible to demonstrate that most cultured white cells have about the same sensitivity to each drug. However, 2 of 3 lines isolated from Burkitt tumor biopsies were totally resistant to prednisolone as was the mouse L1210 leukemia line. A culture derived from a normal individual appeared resistant to 6-mercaptopurine.

Comparison of in vivo and in vitro data has shown that the drug sensitivity of leukemic cells within the patient is similar to that of the cultured leukocytes. However, the short half life of most anti-leukemic drugs in vivo prevents the optimal cell kill achievable in vitro, where drug levels can be maintained as long as necessary. It may, therefore, be possible to predict what modifications of clinical drug regimens will be necessary in order to improve patient response.

Significance:

Intensive anti-leukemic chemotherapy has succeeded in increasing remission rates and prolonging remission, but has failed to produce a significant number of cures. A more thorough understanding of drug action is, therefore, needed to determine whether manipulation of drug regimens may improve the outcome of treatment. Human leukocytes growing in long term culture may serve as a convenient tool for the study of drug-target cell interactions.

Serial No. 4874b

Proposed Course:

Bioassay of additional drugs will be undertaken and in vitro studies of drugs continued. Every effort will be made to complete correlation of clinical data with experimental in vitro observations to permit the early use of the human leukocyte culture model for the design of more efficient anti-leukemic drug regimens.

Serial No. 4875a
1. Viral Biology Branch
2. Microbiology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Interferon Studies with Murine Sarcoma Virus (Moloney)

Previous Serial Number: None

Principal Investigator: Gerald R. Fitzgerald, Ph.D.

Other Investigators: Jennifer Van Brunt, B.S.
Barbara Wayne, B.S.

Cooperating Units: Samuel Baron, M.D., LBV, NIAID
P. S. Sarma, Ph.D., Microbiological Associates

Man Years:

Total:	0.6
Professional:	0.3
Other:	0.3

Project Description

Objectives:

To demonstrate the susceptibility of Moloney Sarcoma Virus (MSV) to interferon action.

Methods Employed:

A high-titered Newcastle disease virus (NDV) of the Herts strain was obtained in allantoic fluids harvested from infected embryonated chicken eggs. An interferon preparation of high potency (10^4 interferon units per ml) was obtained in the serum of adult Swiss mice bled 6 hours after intravenous injection of the virus. Control serum was obtained from mice similarly inoculated with virus-free allantoic fluids. The sera were held at 4°C for 5 days at pH 2.0 to eliminate residual NDV. The properties of the preparations were shown to be specific for interferon by demonstrating the following characteristics: (1) no direct inactivation of NDV; (2) inhibition of vesicular stomatitis virus in infected mouse cell cultures but not in infected chicken embryo cell cultures; (3) no sedimentation of the interfering principle at 100,000 x g for 1 hour; and (4) insensitivity to acid treatment (pH 2.0).

Major Findings:

(1) Mouse serum interferon inhibited Moloney Mouse Sarcoma Virus (MSV) focus formation effectively in both mouse embryo cell cultures and JLS-V9 mouse cell cultures.

(2) Inhibition of MSV focus formation due to interferon activity occurred in both confluent and nonconfluent mouse embryo cell cultures essentially to equal degree and was dose dependent.

(3) Because of the dynamics of MSV focus formation (each focus is apparently the result of re-infection of surrounding cells by virus released from original cell infected), the most effective procedure for demonstrating MSV sensitivity to interferon was to treat cultures with interferon simultaneously with the inoculation of MSV.

(4) Interferon treatment at any time from 24 hours before to 3 hours after MSV inoculation effectively inhibited focus formation. Interferon treatment 8 to 48 hours after MSV inoculation had little or no effect on focus formation.

(5) Cells treated with interferon for 3 hours and then inoculated with MSV were effectively protected against focus formation. Similar cells treated with interferon for three hours and inoculated with MSV 24 hours later were completely unprotected.

(6) Preliminary evidence suggests that interferon treatment also inhibited MSV yield.

Significance:

These results suggest that interferon may play an important role in the outcome of the MSV-host pathological relationship.

Proposed Course:

Work is continuing to answer the following questions: (1) Does MSV induce interferon formation? (2) Are the leukemia viruses susceptible to interferon action? (3) Do the leukemia viruses, under optimum conditions, induce interferon production?

Serial No. 4875b
1. Viral Biology Branch
2. Microbiology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Titration of Mouse Leukemia Viruses (Moloney, Rauscher) By Means of Interference and Helper Activities with Moloney Sarcoma Virus

Previous Serial Number: None

Principal Investigator: Gerald R. Fitzgerald, Ph.D.

Other Investigators: Jennifer Van Brunt, B.S.
Charlotte Blevins, B.S.

Cooperating Units: Dr. Robert A. Manaker, Head, Microbiology Section, NCI

Man Years:

Total:	0.6
Professional:	0.3
Other:	0.3

Project Description

Objectives:

To demonstrate the feasibility of assaying murine leukemia viruses by two methods: interference with the development of foci by Moloney sarcoma virus and helper action in the development of foci by incompetent Moloney sarcoma virus preparations.

Methods Employed:

NIH Swiss mouse embryo cell cultures or JLS-V9 cell cultures were inoculated with dilutions of Rauscher or Moloney leukemia viruses. At various times thereafter the infected cultures were either challenged with Moloney Sarcoma Virus (MSV) or the fluids from the cultures were collected and added as "helper" to MSV-inoculated leukemia-free cell cultures. In the former instance (the interference assay), "competent" MSV was employed. In the latter instance (the helper assay), "incompetent" virus was used. End-point dilutions of leukemia virus were detected in the first instance by the ability of these cell cultures to significantly decrease the number of MSV foci developed in control, leukemia virus-free cultures, and in the second instance by the ability of fluids from these cell cultures to significantly increase the number of MSV foci developed in control, leukemia virus-free cultures.

Major Findings:

Both Rauscher and Moloney leukemia viruses grew progressively in NIH Swiss mouse embryo cell cultures and in JLS-V9 cell cultures, and end-point dilutions of these viruses could be ascertained by the ability of the cells from these cultures to interfere with MSV focus formation and by the ability of the fluids from these cultures to increase significantly MSV focus formation by "incompetent" virus.

Significance:

Two useful and practical methods for assaying leukemia viruses were evaluated. Results are available by these methods in three weeks as opposed to as much as six months for animal titrations.

Proposed Course:

This work has accomplished its purpose and has been terminated.

Serial No. 4875c
1. Viral Biology Branch
2. Microbiology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The Demonstration of a One-Step Growth Cycle for the Murine Sarcoma Virus (Moloney)

Previous Serial Number: None

Principal Investigator: Gerald R. Fitzgerald, Ph.D.

Other Investigator: Barbara Wayne, B.S.

Cooperating Units: S. Baron, M.D., LBV, NIAID

Man Years:

Total:	0.6
Professional:	0.3
Other:	0.3

Project Description

Objectives:

In order to properly design any biochemical experiments with animal viruses it is necessary first to know the time of appearance of new infectious virus, the period of the reproductive cycle during which new virus is formed, whether the virus is spontaneously released, and if so, when. The fulfillment of these requirements constitutes the objective of this project. However, in the case of the Moloney sarcoma agent (MSV), certain problems must first be overcome: (1) Tumor-derived preparations of MSV of a titer sufficiently high to make a single cycle growth study feasible are at present toxic to cells; (2) Accurate determinations of the optimum volume of virus inoculum to cell surface ratio and the optimum virus adsorption time have not been determined. Therefore, the objectives of this study must also include the solution of these preliminary problems.

Methods Employed:

Attempts to prepare a high titer non-toxic MSV preparation consisted of propagation of MSV in roller cultures of mouse embryo and JLS-V9 cell cultures and of concentration of such virus preparations by means of Aquacide (Calbiochem) treatment. MSV adsorption kinetics were studied by measuring virus adsorption in terms of focus formation at various specified times.

Major Findings:

(1) Under the conditions studied, MSV adsorbed to cells at essentially a linear rate for the first 40 minutes. At this time approximately 70% of the virus was adsorbed. By 60 minutes, approximately 90% of the virus was adsorbed; at 2 hours, 99%.

(2) In plate cultures of JLS-V9, mouse cells seeded 24 hours earlier with 5×10^5 cells per 50 mm tissue culture dish and inoculated with 2×10^5 FFU MSV, the titer with optimum amounts of added mouse leukemia "helper" virus was approximately 1×10^5 FFU/ml 48 hours after injection.

(3) Concentration procedures using Aquacide treatment on MSV produced in roller cultures of mouse embryo cells and in roller cultures of JLS-V9 cells effectively raised the titer 10-fold in each case.

Significance:

The results demonstrate the feasibility of preparing a high titer non-toxic MSV preparation which will permit the establishment of a one-step growth cycle of MSV *in vitro*.

Proposed Course:

Work will continue to determine the conditions necessary for production of high titer MSV *in vitro* and to determine a one-step growth cycle for MSV.

Serial No. 4875d
1. Viral Biology Branch
2. Microbiology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: NDV-Induced Interferon Production in Mouse Leukemia Virus-Infected Cells

Previous Serial Number: None

Principal Investigator: Gerald R. Fitzgerald, Ph.D.

Other Investigator: Barbara Wayne, B.S.

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description

Objectives:

It has been reported that Rauscher leukemia virus inhibited the interferon response in mice to Newcastle Disease virus (NDV). It was thought valuable, therefore, to determine whether prior infection with Rauscher virus might inhibit the interferon response of mouse cells to NDV *in vitro*.

Methods Employed:

Rauscher leukemia virus-infected mouse embryo cell cultures and leukemia virus-free mouse embryo cell cultures were inoculated with 10^9 PFU NDV, and cells and fluids were collected at 6, 12, 24, 48, and 72 hours later. These were divided into two parts each, one of which was assayed for NDV infectivity in chick embryo cell cultures, and the other was acid treated and assayed for interferon activity against Vesicular Stomatitis Virus (VSV) plaque formation in mouse embryo cell cultures.

Major Findings:

(1) NDV does not appear to propagate in either Rauscher virus-infected cultures nor in uninfected cultures, although a large amount of the virus did maintain its infectivity for 72 hours (10^5 to 10^6 PFU).

(2) There was no significant difference in the interferon response of mouse cells infected with Rauscher leukemia virus after NDV inoculation as compared to that of uninfected mouse cells after similar challenge with NDV.

Significance:

Rauscher leukemia virus does not appear to inhibit the ability of mouse embryo cells to respond to NDV with interferon production.

Proposed Course:

It is suggested that this avenue of research be abandoned.

Serial No. 4883a
1. Viral Biology Branch
2. Virus Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Culture of Human Lymphoma Cells for the Purpose of Recovering Viruses Which May Be of Etiological Significance

Previous Serial Number: Same

Principal Investigator: Dr. Sarah E. Stewart

Other Investigators: Supporting Staff

Cooperating Units: Mitchell, E.Z.

Man Years:

Total:	2.6
Professional:	0.6
Other:	2.0

Project Description

Work has continued on the virus isolated from the Hodgkin's disease lymph node cell line. It has not been fully characterized, but it was found to fall into the group of paramyxoviruses.

No honors, awards, or publications relating to this project.

Serial No. 4883b
1. Viral Biology Branch
2. Virus Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies With the Mouse Renal Carcinoma Transplanted Tumors to Determine if the Mouse Renal Carcinomas Can Be Induced With a Virus

Previous Serial Number: Same

Principal Investigator: Dr. Sarah E. Stewart

Other Investigators: Dr. Nina Popoff (guest worker)
Dr. A. Claude
Miss Dorothy Calnan
Supporting Staff

Cooperating Units: None

Man Years:

Total:	2.6
Professional:	0.6
Other:	2.0

Project Description

Project is same as described in 1967 project report. Also studies have been initiated with Dr. A. Claude's BALB/c kidney carcinoma.

Results:

Except for an occasional S.C. tumor in the inoculated mice, hamsters and rats, and, generalized leukemias in the BALB/c mice that received the mouse leukemia virus along with the kidney carcinoma extracts, no tumors have developed in a period of 12-14 months.

Cultures of the kidney carcinoma which occurs spontaneously in about 60% of Dr. Claude's BALB/c mice grow readily as cell suspensions. EM studies and animal inoculations studies are in progress.

No honors, awards or publications related to this project.

Serial No. 4883c
1. Viral Biology Branch
2. Virus Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on the Morphological and Immunological Identity of the SL_1 Hamster Brain Passage Virus With the Burkitt Herpes Type Virus.

Previous Serial Number: Same

Principal Investigator: Dr. Sarah E. Stewart

Other Investigators: Mr. B. Lloyd, Jr.

Cooperating Units: None

Man Years:

Total:	2.6
Professional:	0.6
Other:	2.0

Project Description

Work was initiated to determine if the hamster brain passage virus recovered from 3 different lymphoma cell lines and the herpes-type virus of the Burkitt tumor lines are the same agent. This was carried out by inoculating a virus-free lymphoma cell line (SS_3) with the hamster brain passage virus, checking for the presence of virus by electron microscopy after different periods of growth and then determining the antigenic relationship of the two viruses by immunofluorescence tests.

The SS_3 virus-free lymphoma cell line was established from a lymph node biopsy from a mycosis fungoides patient. This cell line was found to be free of virus by Dr. M. Lutzner who supplies us with the biopsy specimen; by Mr. B. Lloyd who examined the controls for the cultures that received the hamster brain passage virus; and by Dr. Nina Popoff who examined these cultures after they received murine type C particles along with uninoculated controls.

This cell line as well as 8 other continuous suspension culture cell lines were lost due to a pseudomonas contamination in the culture medium. None had been frozen in nitrogen, but, we were able to replace them as we had given them previous to the bacterial contamination to another NIH investigator. The SS_3 cultures that had received the hamster brain passage virus metabolized much slower than the uninoculated controls so the culture medium had not been changed in these. Thus, they did not get the bacterial contamination. The immunofluorescence studies were carried out on these cultures but the original controls were no longer available. The SS_3 replacements were thus used as controls. These were shown to have virus. As I believe this was due to a mix-up in the replacements, I felt justified in disregarding these controls. Now, however, we have succeeded in establishing this same SS_3 cell line from cultures that had been frozen in the Revco before the bacterial contamination. The immunofluorescence experiments with the hamster brain passage virus are being repeated.

Honors, Awards and Publications:

1. Stewart, S.E., Glazer, D.E., Stevenson, M.I. and Boliver, J., Jr.: Method for Culture of the Burkitt Herpes-Type Virus and the Antigenic Identity of Three Lines Isolated. Proceedings III^{d} International Symposium on Comparative Leukemia Research, Paris, France, July, 1967. (In press)

2. Stewart, S.E.: Virus Studies in Human Leukemia-Lymphoma: Proceedings Internat. Conference on Leukemia-Lymphoma, 1967. (In press)

3. Stewart, S.E., Glazer, D.E., Ben, T. and Lloyd, B., Jr.: Studies on the Hamster Brain Passage Virus From Human Lymphoma Cultures. J. Nat. Cancer Inst., 40, 2, 423, 1968.

Serial No. 4883d
1. Viral Biology Branch
2. Virus Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on Viruses Recovered From Human Liposarcomas

Previous Serial Number: Same

Principal Investigator: Dr. Sarah E. Stewart

Other Investigators: Dr. Shigeko Nomura and Supporting Staff

Cooperating Units: Mrs. E.Z. Mitchell

Man Years:

Total: 2.6
Professional: 0.6
Other: 2.0

Project Description

Two liposarcoma specimens R000614 and R000718 which had been frozen at -190°C for 3 years and 2-1/2 years respectively were grown out on tantalum mesh grids in T30 flasks (1967 Annual Report). Viruses were isolated from the culture supernates from both specimens by inoculating WI-38 cells with the supernatant fluids.

Both cultures appear to carry more than one virus. This has been substantiated both by electron microscopy and by immunologic studies.

Complement-fixation tests done by Mr. H. Turner (courtesy D. K. McIntosh) using guinea pig sera prepared against the 614 virus when tested against known antigens gave the following results:

Guinea Pig^{+} Serum 614 Complement-Fixation Titration --

Antigens	1/20	1/40	1/80	1/160	1/320	1/640	1/1280
Measles*	4+	4+	4+	4+	4+	4+	2+
RS Pool*	4+	4+	4+	4+	4+	2+	1±
Rubella	4+	4+	4+	4+	4+	4+	1±
SV5	4+	4+	4+	4+	4+	4+	3+

*Virus grown in human cells

+Normal GP sera have antibody to SV5 but usually only about 1/40. Parainfluenzas 1,2,3, and 4 were all negative.

The above indicates that our 614 virus may be a mixture of rubella and SV5.

Serum neutralization tests carried out by Dr. A. Vargosko against 614 virus gave the following results:

Viruses 614 and prototype parainfluenza 2 were tested against parainfluenza type 2 serum (rabbit). Virus 614 was inhibited by a 1:20 dilution of serum. There was no inhibition at 1:40. Prototype parainfluenza 2 was not inhibited by the homologous serum. Specific serum prepared against 614 indicated a titer greater than 1:160; against prototype para 2, 1:10 only.

No relationship indicated between 614 and the following antisera: RS, and mumps.

Our typing results as found by hemadsorption inhibition and by serum neutralization in tissue culture indicate that we have isolated an SV5-like virus from each of the human sarcomas.

Although this virus is neutralized by SV5 antiserum it appears to be different morphologically from the simian virus. It also differs from it in that it causes an encephalitis in hamsters when inoculated I.C. while SV5 is nonpathogenic when injected in the same manner.

Reciprocal cross neutralization tests are shown in the Tables:

Relationship of 614, 718 viruses and other myxoviruses of the parainfluenza group by neutralization tests --

Antiserum	Reciprocal Neutralizing Antibody Titer to Indicated Antigens		
	614 - 28	718 - 29	SV5
Parainfluenza 1	8	8	
Parainfluenza 2	8	8	
Parainfluenza 3	8	8	8
RS	8	8	
SV5	2048	2048	4096
Measles	32	32	
Rubella	8	8	

Cross Neutralization Test

Antiserum	Reciprocal Neutralizing Antibody Titer to Indicated Antigens		
	614 - 28	718 - 28	SV5
SV5	2048	2048	4096
718 - 28	1024	1024	256

From the neutralization tests obtained by us and the others it appears that the liposarcomas had more than one virus initially but that on continued culture the SV5-related agent may have masked the others. Monkey kidney cells which were generally implicated in those reports on SV5 isolations from human tissues were not used in our isolations.

More frozen culture specimens including 614 and 718 liposarcomas were received from Dr. DePue in February 1968. The 614 and 718 specimens were divided and one half of each was given to Dr. S. Nomura for culture and virus isolations. Both her cultures and those which I am carrying are again yielding hemadsorbing viruses. SV5 antiserum does not appear to inhibit all the new isolates. The 614 virus cultures without the SV5 antiserum have a hemadsorbing virus, with the SV5 antiserum a non-hemadsorbing virus; while the 718 virus cultures have a hemadsorbing agent in cultures both with and without SV5 antiserum. Electron microscopy studies have not been done.

No honors, awards, or publications related to this project.

SUMMARY REPORT

VIRAL LEUKEMIA AND LYMPHOMA BRANCH

July 1, 1967 - June 30, 1968

The major objectives of the Viral Leukemia and Lymphoma Branch are (a) to determine whether viruses are etiologically associated with at least some human neoplasms, and (b) to develop effective means for the prevention and control of these diseases, when such etiologic agents are found.

The Branch seeks solutions to the problems of virus-induced neoplasia in a coordinated scientific attack by Groups or Sections composed of Senior Investigators of different scientific disciplines.

The Section of Ultrastructural Studies employs electron microscopy for the examination of neoplastic tissues and isolated viruses. The Section of Molecular Virology studies the quantitative aspects of cell-virus interactions and seeks elucidation of the phenomena of cancer at the molecular level. The Section of Immunology explores the immunological aspects of viral oncology both in terms of viral and cellular antigens and of humoral and cell associated antibodies. The Section of Virus Disease and Modification employs and expands current concepts and develops new concepts of immunology and chemotherapy for the control of virus-induced tumors. The Section of Viral Leukemia seeks an understanding of the relationships of viruses to cancer in the intact animal and in selected tissue culture systems with a view to the ultimate application of the findings to the control of the human disease.

Significant advances were made within the Branch which contribute to the understanding of viral leukemia and sarcoma. Studies on the interrelationship of virus-induced leukemias and sarcomas received considerable emphasis since the understanding of this interrelationship holds high potential for the detection of a possible human tumor virus and the prevention or control of the disease as it occurs in man.

Quantitative "in vitro" studies on the behavior of the murine sarcoma virus, utilizing the focus-forming assay, confirmed the earlier observation of the defectiveness of this virus. The present data also indicated the existençe of a competent sarcoma virion. The studies of the Molecular Virology Section indicated that this competent virion was a temporary zygotic hybrid of a sarcoma virion and a leukemia virion. This finding is noteworthy in that it represents the first observation of an RNA-containing hybrid tumor virus.

The elucidation of the composition of murine sarcoma virus stocks in terms of a 3-fold mixture of competent sarcoma virus, defective sarcoma virus and excess leukemia virus permitted the development of a scheme in which any murine sarcoma virus of defined composition can be employed in tissue culture systems as an indicator for the detection of exogenous leukemia virus. Thus, there is now available a rapid relatively easy assay of murine leukemia viruses. This assay should facilitate the development of the quantitative cellular biology of murine leukemia viruses and an understanding of the molecular processes associated with virus-cell interactions.

The pathogenesis of tumor development, progeny-virus production, and of antibody development following inoculation of susceptible mice with Moloney Sarcoma Virus (MSV) were intensively studied by members of the Viral Leukemia Section. It is of interest that although tumors induced by this virus in weanling mice usually regress, high levels of infectious virus could be recovered from the sites of inoculation, the plasmas, spleens and livers of the infected animals. It was observed that nucleated spleen cells from animals with regressed tumors could suppress growth of transplanted primary virus-induced tumor cells but these immune cells had no effect on transplanted metastatic cells. The metastatic cells grew when transplanted into immunized isogenic hosts, however, transplanted primary tumor cells were rejected by mice similarly conditioned. These findings strongly suggest that metastatic tumor cells differ significantly from primary tumor cells in their susceptibility to control by host immune mechanisms.

This group of investigators have continued their human leukemia virus studies through the investigation of the following cases:

(1) Simultaneous occurrence of the same cell type of acute leukemia in next door neighbors.

(2) Occurrence of Acute Lymphocytic Leukemia (ALL) in two cousins in the same household.

(3) Investigation of a child with ALL and his normal twin.

(4) Occurrence of Acute Myeloid Leukemia in a father and Rhabdomyosarcoma in his one-year-old son.

(5) Simultaneous occurrence of lymphosarcoma in a patient and his pet.

Laboratory studies included electron microscopic review of the plasma and tumor specimens, chromosome analyses and establishment of long-term lymphocyte cultures. Sera is stored at the Communicable Disease Center.

The role of the host immune system in the regression of primary MSV tumors has been of interest to investigators in the Virus and Disease Modification Section. Their studies have emphasized the finding that administration of selected serums inhibit the regression of tumors in adult animals.

It was observed that sarcoma-induction and tumor survival was increased by the inoculation of high dilutions of any of several murine leukemia viruses.

The tumors which developed in the adult "conditioned" animals did not regress but progressed to large size and killed the animals. These observations are noteworthy in that they describe the role of leukemia viruses in the induction of sarcomas which otherwise do not become clinically manifest.

Early studies by this and other groups on the protection of mice against either virus-induced leukemias or sarcomas with forminalized viral vaccines were considerably extended. Immunization with Friend leukemia vaccine protected mice against challenge with infectious Friend and Rauscher leukemia viruses but not against challenge with the Moloney leukemia virus. Immunization with Moloney leukemia virus vaccine protected mice against challenge with Moloney leukemia virus and Moloney sarcoma virus but not against challenge by the Rauscher or Friend viruses. These studies are being continued in an effort to define the composition of a possible polyvalent vaccine for maximum protection.

The Immunology Section has emphasized studies in murine leukemia which might be applicable to problems of diagnosis, therapy, or prevention of human leukemia in man.

Thus, passive hemagglutination appears to provide a sensitive method for the detection of antibody to murine leukemia viruses in antiserum prepared in several species. A variation of this test in which it is essentially employed as an inhibition test appears to provide the most sensitive assay for murine leukemia virus antigen available to date.

The micro-immunodiffusion method has been used to delineate the various antigens of a murine leukemia virion from each of several virus strains. It has been shown that most if not all of the murine leukemia virus strains share a nucleoid antigen which is either identical or strongly cross-reactive.

This antigen can also be demonstrated by hemagglutination inhibition and complement fixation tests. The immunodiffusion method has been used to identify the HHLV-1 virus of Herbut, as a virus of murine origin. This agent was recovered from mice inoculated with human leukemic material.

The techniques of immunodiffusion and passive hemagglutination was applied to studies of the herpes virus isolate from Burkitt lymphoma cell lines. Major observations resulting from these studies include the following:

1. A large percentage of adults, regardless of their experience with leukemia or lymphoma, contain antibody to the virus isolated from the P-3 cell line.

2. Viruses isolated from several cell lines are either identical or strongly cross-reactive.

3. A Rhesus antibody prepared against the C-type virus from an acute myelocytic leukemia, a Rhesus antiserum prepared against the herpes virus from the P-3 cell lines, and a serum from a Burkitt lymphoma

patient show a line of identity or strong cross reactivity when virus from the P-3 cell line is used as antigen.

The objectives of the Ultrastructure Studies Section are to utilize the technique of electron microscopy to describe and elucidate the ultrastructure of malignant cells, and to further define the relationship between the presence of viruses and the existence of neoplasia. Electron microscopic observations are integrated with biochemical and immunological findings in an effort to understand carcinogenesis and cellular differentiation.

Present efforts in collaboration with Dr. E. L. Kuff are directed toward identification and characterization of the proteins in the inner shell of the Type A particles associated with certain plasma cell tumors. Purified preparations of these particles are being tested serologically in order to determine the possible relationships to the intracytoplasmic A-particles which are seen in mouse mammary tumors.

Ultrastructural studies of the mineral oil-induced and plastic disc-induced mouse plasma cell tumors are continuing. Preliminary results indicate that A-particles may be present in various cell types in the peritoneal cavity prior to the overt development of tumors.

Recently studies have been initiated with Drs. Richard Asofsky and Mark Mandel to determine the differentiation of immunocompetent cells in virus-infected mouse systems. This has been possible because of techniques utilizing prolonged drainage of the thoracic duct. The cell types in the thoracic duct lymph have been classified on an ultrastructural basis.

A collaborative study on the localization of viral antigens of Tween-ether treated Rauscher virus has been partially completed. The treated preparations were placed on a sucrose density gradient and the gradient fractions were evaluated by immunodiffusion, hemagglutination inhibition and electron microscopy.

Studies of the Novikoff hepatoma have revealed the presence of a C-type particle in the tumor, but attempts to propagate virus from this material have thus far been unsuccessful.

Proposed Course of Effort Within the Viral Leukemia and Lymphoma Branch:

The research activities of the Branch will be continued along the lines described with a view to the deliniation of methods for the detection and characterization of viruses of possible etiological significance to human neoplasia.

It is apparent that the greatest efforts of the investigators within this Branch are directed toward a more complete understanding of the biology, immunology, and biochemistry of certain RNA tumor viruses and the induced neoplasms. To broaden the scientific base of the Branch, to develop competence in and understanding of the entire picture of oncogenesis as as it occurs in animals and man, strong consideration is being given to the creation of a research section within the Viral Leukemia and Lymphoma Branch which would be concerned with the study of the DNA tumor viruses and the associated neoplasms.

The collaborative research studies with the Medicine and Surgery Branches of the National Cancer Institute will be extended. It is considered that such studies will permit clinical personnel to more fully appreciate the nature of basic research, the approaches and possible contributions of tumor virology to the management of tumor patients. Such studies will develop an access to selected patient material for virology studies by the personnel of this Branch and contribute to a more complete understanding of the nature of cancer as it occurs in man. These collaborative efforts will allow a more natural orientation of basic studies to the control or prevention of human neoplasia.

Other Activities of the Branch

During the reporting period, senior investigators of the Branch published a total of 38 papers which covered various aspects of the broad area of Viral Oncology. Members of the Branch presented by invitation over 43 lectures to research groups in this country and abroad.

For the purpose of consultation or training, sections of the Branch entertained representatives from every major research institution in this country. In addition, Branch members were visited by senior representatives of universitites, private research and commercial groups from the following countries: England, Scotland, India, Australia, Germany, France, Italy, Russia, Czechoslovakia, Hungary, Denmark, Sweden, The Netherlands, Japan, Argentina, Chile and Uruguay.

Although the "in house" basic research efforts are of prime interest to members of this Branch, a majority of the investigators contribute up to eighty percent of their time in support of the Special Virus-Leukemia Program of the National Cancer Institute. The members serve as Chairmen, Vice-Chairmen, Work Group members or Secretaries of the major segments of the Program. They provide scientific guidance as Project or Assistant Project Officers on research contracts supported by the Leukemia Program. Two of the three subgroups of the Program are chaired by senior researchers of this Branch. Dr. Mary Fink as Chairman of the Immunology subgroup, has assumed responsibility for coordinating the Program immunological and serological activities as related to studies of known human viruses and with candidate agents isolated from human leukemia and lymphoma materials. Dr. Timothy E. O'Connor as Chairman of the subgroup on Biophysics is responsible for planning and coordinating contract studies concerned with the biochemical and biophysical characterization of known animal tumor viruses and of candidate viruses recovered from man.

Serial No. NCI - 4812
1. Viral Leukemia & Lymphoma Branch, OASDVO, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: (A) Investigation of the possible role of arthropods in the transmission of tumor viruses.

(B) Supervision of leukemia transmission studies in the dog.

Previous Serial Number: 4882

Principal Investigator: George J. Burton, Ph.D.

Other Investigators: None

Cooperating Units: (A) None

(B) Michigan Department of Health

Man Years
Total: 1
Professional: 1
Other: 0

Project Description

Objectives:

Project A

1. To advise the Project Director, Dr. Robert G. Fischer, Chairman, Dept. of Microbiology, University of North Dakota, Grand Forks, North Dakota, in the entomological aspects of Contract PH43-66-8 entitled "Insects, ticks, and mites as possible experimental and natural vectors of murine and avian tumor viruses."

2. To assist in procuring colonies of arthropods for the project, including mosquitoes, conenose bugs, beetles, biting flies, ticks, mites and bedbugs which are to be utilized in attempts to transmit Rauscher and Friend murine leukemia viruses, Moloney sarcoma virus, avian reticuloendotheliosis virus (Twiehaus strain), and any other viruses which may be designated.

3. To train project staff in breeding, feeding, and maintenance of the various arthropods involved.

4. To train contract personnel in handling and transferring arhtropod species, so as to avoid introducing mortality factors through manipulation of specimens.

5. To train contract personnel in anatomical and physiological aspects of the experimental arthropods, and in techniques of dissecting them.

6. To seek out literature and published studies pertaining to the species of arthropods utilized, and to obtain and furnish reprints to contract personnel, including translations of relevant papers in foreign languages.

7. To contact other scientists who are engaged in investigations relevant to the project, and to arrange for visitation of contract personnel to their laboratories.

8. To assist contract personnel in setting up, or arranging for, special laboratory apparatus and procedures for studying viruses within arthropod tissues, such as tissue culture of arthropod cells, flourescent antibody studies, and electron microscopy of infected arthropods and their components.

9. To assist in procurement of animals, birds, viruses and equipment to be used in the studies.

10. To assist in recruitment of entomological personnel for the contract.

11. To act as liaison officer between the Project Director and professional and administrative personnel at NIH, and to assist in processing and expediting requests from the Project Director.

12. To advise in the selection of entomological equipment and apparatus for the project.

Project B

1. To advise Dr. Gabel H. Conner, Project Director and Professor of Veterinary Surgery and Medicine at Michigan State University, East Lansing, Michigan, as necessary, in carrying out the objectives of Contract PH43-65-100, which is entitled "Leukemia transmission studies in the dog."

2. To visit the project site at intervals, including the subcontractor, the Michigan Dept. of Health, in order to review the progress of the work being done under the contract, and ensure that the aims and purposes of the contract are being fulfilled without irrelevant or unproductive investigations.

3. To provide any necessary guidance and assistance to contract personnel in the performance of their duties.

4. To act as liaison officer between the Project Director and professional and administrative personnel at NIH, expediting all administrative and technical matters, and assisting in solving any problems which may arise.

Methods Employed:

Project A.

As the Project Officer and non-resident entomologist, an average of three days per month has been spent at the project site at the University of North Dakota, in order to give entomological advice, assistance, and training to contract personnel, and to check on the correctness of procedures which had been previously outlined. Together with the Project Director, experiments were planned in feeding certain species of arthropods on viremic animals or material, results were assessed, and experimental designs changed as necessary.

The other personnel on the contract include: 1 supervisor, 2 graduate students, 2 research technologists, 1 entomologist who joined in 1968, and 1 insectary attendant. Training was given in the following: Construction and use of various arthropod-holding cages, dissecting instruments, collecting tubes, and aspirators; construction of feeding membranes and other artificial feeding devices; dissecting and insectary techniques; identification of internal arthropod organs; extraction of hemolymph; techniques of natural feeding on viremic mice and chicks; and recognition of the various stages, instars, and sexes of the arthropods used.

There have been numerous backstopping activities and procedures which have been necessary to make this project function successfully, since there are many types and species of arthropods used, and these must be renewed from time to time. The mosquitoes *Aedes aegypti*, *Culex pipiens quinquefasciatus*, *Culex pipiens pipiens*, and *Anopheles quadrimaculatus* are relatively easy to obtain and breed in the insectary and environmental chambers. Others, however, such as bedbugs, conenose bugs, mites, and ticks, have been difficult to procure within the United States, except for one species of conenose bug. Constant sources of most of the arthropods used are very few in number, since the supplier often needs them for his own studies. Usually only a single shipment of 10 to 30 specimens can be obtained from a source. Breeding of the arthropods at the University of North Dakota has been necessary in order to have them available when needed. Through personal contacts, however, certain species have been replenished, thus assuring continuation of the transmission experiments.

Following location of the arthropod suppliers, it has been necessary to obtain permits from certain U.S. government agencies. For each interstate shipment, a permit must be obtained from the Animal Health Division of the U.S.

Dept. of Agriculture in Hyattsville, Maryland. For shipments from foreign countries directly to the University of North Dakota, a permit must be obtained from the Division of Foreign Quarantines of the USPHS. For shipments from foreign sources directly to Dr. Burton at NIH, entry into the U.S.A. is allowed under a blanket authorization awarded specifically to NIH by the Division of Foreign Quarantines, after which the arthropods must be reshipped under an interstate permit to the University of North Dakota.

The arthropods which were procured during fiscal year 1968 were: MOSQUITOES: *Aedes aegypti* (Linnaeus) - strains from Bangkok, U.S.A., Cameroun, Ivory Coast, and Nigeria; *Culex pipiens quinquefasciatus* Say; *Culex pipiens pipiens* Linnaeus, and *Anopheles quadrimaculatus* Say. TICKS: *Rhipicephalus sanguineus* (Latreille), *Ixodes ricinis* (Linnaeus), *Argas persicus* (Oken), and *Ornithodorus moubata* (Murray). BEDBUGS: *Cimex lectularius* Linnaeus, *Cimex hemipterus* (Fabricius). CONENOSE BUGS: *Triatoma infestans* (Klug), and *Rhodnius prolixus* Stal. Sources were found in the U.S.A., Columbia, Czechoslovakia, Switzerland, England, Canada and Jamaica.

Among the general procedures that have been set up for studying virus transmission by the arthropods concerned are the following:

1. Feeding of the arthropods on virus-infected tissue, viral suspension, or viremic mice or chicks, followed by holding for predetermined time intervals, homogenization of the entire arthropods or of their viscera in a pool, and injection of the homogenate into healthy mice or chicks which are subsequently checked for disease symptoms.

2. Permitting the arthropod species to take a partial, infected blood meal from a viremic mouse or chick, which is suddenly removed and replaced immediately with a healthy donor upon which the blood meal is completed, thus testing for possible mechanical transmission of virus via an interrupted blood meal. Results are compared with those obtained by using infected, fine "minuten" pins to effect transmission of the disease from a viremic mouse, chick, or suspension to a healthy mouse or chick.

3. Permitting the arthropod species to feed on a viremic mouse or chick to repletion, holding them for predetermined time intervals, and then refeeding them on a healthy donor in order to determine whether biological transmission of the disease can occur via the salivary glands, as a result of natural feeding activities.

4. Placing viremic mice (or chicks) and normal mice (or chicks) into a cage together with suitable numbers of the test arthropod species, in order to see whether this free association will result in transmission of the disease to healthy mice (or chicks) via a natural series of events.

5. Feeding the infected arthropod to mice, chicks, or chickens in order to test for transmission of the disease through ingestion of the arthropod.

6. Testing of feces of arthropods which have ingested virus, and after varying time intervals determining the viability of the virus in the excreta. This is followed by attempted transmission of the disease by rubbing the infected feces into normal and scarified skin of a mouse or chick.

7. Testing of eggs of the infected arthropod for virus, in order to determine whether transovarian transmission of the virus can occur.

8. Testing individual portions, tissues, organs, secretions, and excretions of the infected arthropod, such as head, thorax, abdomen, hemolymph, saliva, salivary glands, and tick coxal fluid, for the presence and multiplication of the ingested virus, and comparison of findings with results obtained in biological transmission experiments.

9. Carrying out flourescent antibody and electron microscopic studies on arthropods which have ingested virus during transmission experiments.

Much time has been devoted to checking pertinent journals in English and foreign languages at the Clinical Center Library, NIH, and at the National Library of Medicine, for research studies which are relevant to the species of arthropods used in the project. These have included methods of breeding or colonizing in the laboratory; anatomy, physiology, and histology of the various species; tissue culture of arthropod tissues; electron microscopy of tumor viruses; flourescent antibody studies on viruses in arthropods; and studies on other types of viruses in the species of arthropods untilized. Persons who have published pertinent papers have been contacted to determine whether they are currently engaged in such studies, and arrangements have been made for contract personnel to visit their laboratory, especially for studying tissue culture, flourescent antibody, and rearing techniques. Electron microscopy of infected arthropods will be undertaken in cases of biological transmission, or where there has been extended virus viability within the arthropod without biological transmission. Cytopathological effects on arthropod organs will also be determined.

While engaged in my administrative and backstopping duties at NIH, purchase requisitions, travel requests, and other official matters have been expedited through the various NCI offices so as to minimize delays in receipt of equipment and in receiving replies to official requests made by the Project Director. The latter and the project supervisor have also visited NIH to discuss the work with professional and administrative personnel there.

Project B.

During visits to the dog leukemia transmission project site at Michigan State University, East Lansing, Michigan, and to the subcontractor, Michigan Department of Health in Lansing, the progress made on the project has been reviewed with contract personnel. Consultations have been held with the Project

Director, Dr. Gabel H. Conner; with the Associate Director, Dr. George H. Anderson; with the two virologists, Dr. Ronald W. Hinz (MSU) and Dr. J. R. Mitchell (MDH); and with the Colony Veterinarian, Dr. J.A. Moore. Various problems have been reviewed and solutions effected in accordance with existing circumstances.

. The Dog Leukemia Transmission Building, which is on the extensive Michigan State University campus, has facilities for containment of 160 beagle dogs inocualted with leukemic and known viral material. After 9 months the inoculated dogs are transferred to the long-term holding facility, the H-Bar-B Kennels in Essexville, Michigan. This is situated about 75 miles northeast of the Michigan State University campus, and has facilities for 450 dogs, including those used for breeding. The holding time will range from 18 months to 6 or 7 years postinoculation.

The main objective of the project has been the induction of lymphoma or other neoplasia in the dog following inoculation with viral-associated or viral suspected material of leukemic origin, and to relate the results to the etiology of human leukemia. Leukemic and lymphomatous material of canine, human, avian, or murine origin are inoculated into colostrum-deprived, neonatal beagle dogs obtained by Caesarian section. These dogs are kept in the biohazard-controlled Leukemia Transmission Building for about 9 months, after which they are transferred to the long-term holding facility. During their containment, they undergo daily observation, routine physical examinations, determination of hematological and coprological status, and diagnostic surgical procedures as necessary. Electron microscopy is carried out on leukemic tissues and organs or other material removed from inoculated and outside dogs, in order to search for viruses. Cultures of Burkitt lymphoma cells, dog lymphoma cells, and primary cultures of dog embryo cells are routinely inoculated with cell-free extracts of tumor tissue in an attempt to isolate and recover oncogenic agents; also, serological, immuno-electrophoretic and immuno-diffusion studies are carried out on sera collected from dogs with spontaneous lymphosarcoma and from dogs which have been inoculated at MSU with leukemogenic viruses and lymphomatous tissues and extracts. Virus isolation is also attempted, with the aid of candidate helper viruses especially Moloney sarcoma virus. Related studies are also carried out on dogs having spontaneous lymphoma.

Major Findings:

Project A.

1. Virus viability. To date the viability of various tumor viruses has been determined in adults and late instar nymphs (as applicable) of the following arthropods: conenose bugs - Triatoma infestans and Rhodnius prolixus; mosquito - Aedes aegypti (Bangkok strain), soft tick - Argas persicus, and scavenger tenebrionid, beetle - Alphitobius diaperinus. This has been done by following the first general procedure listed above, i.e. feeding a group of arthropods on

viremic mice or chicks, holding them for predetermined time intervals, homogenizing the entire arthropods, and injection of the diluted homogenate into healthy recipients which are subsequently checked for possible disease. Results given below are based on this procedure, except as otherwise indicated for hemolymph, feces, eggs, or larvae:

Tumor virus	Arthropod species	Virus Viability in Days
Rauscher murine leukemia virus	*Triatoma infestans*	15 days
	T. infestans hemolymph	3 days
	T. infestans feces	1 day
	Rhodnius prolixus	26 days
	Aedes aegypti	0 days
Friend murine leukemia virus	*Triatoma infestans*	20 days
	Rhodnius prolixus	1 day
	Aedes aegypti	1 day
Moloney sarcoma virus	*Triatoma infestans*	4 days
	Rhodnius prolixus	6 days
	Aedes aegypti	0 days
	A. aegypti eggs	0 days
	A. aegypti larvae	0 days
	Alphitobius diaperinus	1 day
Avian reticulo-endotheliosis virus (Twiehaus strain)	*Triatoma infestans*	3 days
	Rhodnius prolixus	12 days
	Argas persicus	6 days

These results indicate that the vector, *Aedes aegypti* cannot maintain any of the leukemogenic viruses tested, and that conenose bugs are good incubators of tumor viruses.

2. Mechanical transmission by interrupted feeding. This procedure involved permitting the arthropod to feed partially on a viremic donor, suddenly withdrawing the donor, and replacing it immediately with a healthy recipient on which the blood meal was completed. All experiments to date with all viruses and all the arthropods listed here have been negative.

3. Mechanical transmission using minuten pins. It was found that three of the viruses listed above could be transmitted by pin pricks as follows:

Moloney sarcoma virus	4 pin pricks,	first into leg tumor of diseased mouse, then into leg of healthy recipient.
Friend leukemia virus	1 pin prick,	first into blood of viremic mouse, then into skin of healthy recipient.
Reticulo-endotheliosis virus (Twiehaus strain)	6 pin pricks,	first into infected plasma, then into skin of healthy chick

These data, when compared, indicate that probably very few viral particles are necessary to transmit Friend leukemia.

4. Biological transmission. All biological transmission experiments following the natural course of feeding, i.e. a full blood meal on a viremic mouse or chick, then a subsequent full blood meal on a healthy mouse or chick, have been negative, except for one experiment involving Friend virus as follows: Five cages of 5 Triatoma infestans were fed to completion of Friend leukemia infected mice, were then maintained for 20 days in an environmental chamber, and then allowed to feed again on healthy mice. Three out of 4 of the latter mice developed the myeloproliferative form of the disease. It is probable here, too, that few viral particles are involved in transmission of Friend leukemia. Electron microscopic study would be valuable in determination of the presence and number of viral particles within various organs of this species, following infection by feeding on a viremic mouse.

Project B

1. There have been relatively few significant findings among 329 inoculated neonatal dogs; (a) 3 malignant lymphoma (ML) deaths have occurred from inoculated whole canine lymphoma cells, and 1 malignant lymphoma death from extract, in less than 90 days; (b) inocula of human and avian origin have yielded negative results; (c) inocula of murine origin have yielded mainly negative results, except in one dog which developed reticulum-cell type of malignant lymphoma 4 months postinoculation with rat sarcoma virus, and subsequent regression to neoplasia.

2. The viral findings on this project have been: (a) isolation of herpes-like MDH-1 virus in canine thymus cells that had been inoculated with

P3-J Burkitt cells superinfected with Moloney sarcoma virus; (b) isolation of a canine herpesvirus from a ML lymph node; (c) demonstration of herpes-like particles in 21 (23.3%) out of 90 dogs with spontaneous ML; (d) demonstration of C-type particles in 5 cases of spontaneous malignant lymphoma; (e) demonstration of C-type particles in vitro in transformed dog ML cells; (f) demonstration of C-type particles in vitro from a lymph node of induced canine mast cell leukemia, (g) demonstration of budding viral particles in a cell line derived from a spontaneous canine fibrosarcoma; and (h) demonstration of crystalline arrays in EM preparations made from most dogs having malignant lymphoma, and from some dogs having distemper.

Significance to Biomedical Research and the Program of the Institute:

Project A. Hematophagous arthropods such as mosquitoes, bedbugs, biting flies, fleas, conenose bugs, ticks, and mites, may suck blood from man, domestic animals, and domestic birds, all of which may develop forms of leukemia. It is therefore important to determine whether such arthropods can maintain and transmit leukemogenic viruses to animals, birds and man. For example , the conenose bug Triatoma infestans (family Reduviidae, order Hemiptera) feeds on man, poultry, and rodents in nature. Non-hematophagous insects, such as scavenger beetles which feed on dead poultry (i.e. Alphitobius diaperinus) may also be able to maintain leukemogenic viruses which may be present in excreta, or which may be taken up when feeding on diseased carcases. Such infected beetles may be ingested by healthy poultry, which may possibly become infected in turn. Some of these studies may have application to the theory of transmission of Burkitt's tumor by bloodsucking arthropods.

Project B. By isolating, identifying, and studying viruses found in both canine and human leukemia including lymphoma, similarities may emerge which may link the disease in man with that of the dog.

Proposed Course:

Project A. This project will be continued for an indefinite number of years until a sufficient number of experiments will have been carried out with many species of different types of arthropods, enabling definite conclusions to be reached as to their relative importance in maintaining and transmitting leukemogenic viruses in nature, the possible involvement of man, and the relationship, if any, of arthropods to the etiology of human leukemia.

Project B. The study of canine leukemia has been considered relevant to the overall investigations concerning the relationships of domestic animal leukemia to human leukemia, therefore this study will continue indefinitely, although on a delimited, reduced scale, so that a final decision can be reached as to the possible involvement of the dog in the etiology of human leukemia.

Honors and Awards: None

Publications: None

Serial No. NCI-4823
1. Viral Leukemia & Lymphoma Branch
Branch, OASDVO, Etiology
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The application of immunological concepts to the study of tumor viruses, to include methods for detection of viruses as etiological agents of malignancy; and the diagnosis, therapy, and prophylaxis of viral neoplasia by immunological methods.

Principal Investigator: Mary A. Fink, Ph.D.

Other Investigators: Louis R. Sibal, Ph.D.
Max H. Cohen, M.D.

Cooperating Units: Inside NIH:

Dr. John Moloney
Dr. Timothy O'Connor
Dr. Nelson Wivel
Dr. Janet Hartley (NIAID)
Laboratory Aids Branch

Outside NIH:

Dr. W. Feller, Georgetown University, Washington D.C.
Dr. P. Herbut, Jefferson Medical College, Phila., Pa.
Dr. M. Mizell, Tulane University, New Orleans, La.
Dr. G. King, Trinity College, Washington D.C.
Dr. C. Burger, State University of New York, Syracuse, N.Y.
Dr. R. Lewis, New England Medical Center, Boston, Mass.
Dr. J. Mitchell, Michigan State Dept. of Health, East Lansing, Michigan
Dr. H. Bowman, State University of New York, Buffalo, N.Y.

Man Years: (July 1, 1967 through June 30, 1968)

Total: 5
Professional: 5/6 (Two-thirds of principal investigator's time is devoted to the Special Virus-Leukemia Program as Chairman of the Testing and Monitoring Segment; Chairman of the Immunology Subgroup, and as Project Office on several contracts.)
Other: 4-1/6

Serial No. NCI-4823

Project Description

Objectives:

The overall objectives are twofold:

1. To develop new, or to adapt current methods for the detection and identification of viruses causally related to malignancies.

2. To study the basic immune response of the host to a leukemia virus.

The principal model system used in these studies has been a murine leukemia virus (Rauscher). Various studies have also been conducted with other strains of murine leukemia viruses, avian leukosis viruses, and the mammary tumor agent. Any finding made in a model system is immediately exploited, if feasible, in an attempt to apply it to problems of diagnosis, therapy, or prophylaxis of viral neoplasia in man.

Methods Employed:

Immunofluorescence (FA): During the last year, a contract laboratory was established at Bionetics Research Laboratories (PH43-67-661), chiefly for the performance of various immunofluorescent techniques. This has relieved our laboratory of some of the routine work performed for various investigators within NIH or the Special Virus-Leukemia Program (SVLP). A limited amount of FA work has been done in-house this year, including testing the marrow of a case of acute lymphocytic leukemia which has been in remission for 13 years. Essentially negative results were obtained with the anti-"C-type" antiserum. This patient was sent to the Clinical Center by Dr. Herbert Bowman of Buffalo, New York, after the Leukemia/Lymphoma Symposium in October in Ann Arbor, Michigan.

No further attempts have been made at this time to apply immunofluorescence to a study of murine leukemias.

Immunodiffusion:

A. Murine Leukemia -- Micro-immunodiffusion has continued to be a reliable tool in studying leukemia of rodents and other species. Our original work using the Rauscher strain of murine leukemia virus has been amplified to include several other strains. Through contract PH43-64-533 murine leukemia viruses prepared in SPF, BALB/c mice have been obtained in pilot study quantity from animals infected with Friend, Moloney, Rauscher, Gross, Breyere-Moloney, C-60, Buffett, and Rich. Two other viruses being studied are the Herbut murine leukemia virus, HHLV-1, and the Kaplan virus. Preliminary evidence indicates that most, if not all (Buffett ?)

share at least one antigen which is either identical or is strongly cross reactive. From studies involving physical separation of the antigens of the virion, this antigen appears to be in the inner core. Continuation of the physio-chemical separation and identification of antigens and a study of their physical location within the virion progressed somewhat this year due to the loan of an expert technician from Dr. Moloney's laboratory. One consequence of this preliminary work was the isolation of the nucleoid antigen as described by O'Connor. This material has been tested by electron microscopy (Wivel), passive hemagglutination inhibition (HAI) (Sibal), immunodiffusion (Fink), and complement fixation (CF) (Hartley). The HAI detects at least one log more virus than CF. Separation and isolation of the outer coat proteins by the method described by O'Connor has been unsuccessful and other methods will be tried.

In immunodiffusion, mouse antibody reacts similarly to Rhesus monkey antibody in that the gamma-G globulin is precipitable with virus, but gamma-M does not form a visible precipitate.

The technique of immunodiffusion was applied to the identification of a virus isolated by Dr. Peter Herbut from mice which had been inoculated with human leukemic material. Both the mouse derived virus and the original inoculum were tested in the immunodiffusion system. The virus was clearly identified as being murine in origin. Confirmation of the immunodiffusion findings was made by HAI (Sibal) and EM (Wivel).

B. Human Leukemia -- Immunodiffusion studies using Burkitt-lymphoma-derived-herpes virus and human serums , as described in the last annual report, were continued. Various serums were tested with antigen from the P-3 line to find very little difference in the reactivity of serums from leukemia, lymphoma, or controls. This, in general, agrees with the data of the Henles' using immunofluorescence. It is in disagreement with that of Old, who also used immunodiffusion to find reactivity only in the serum of Burkitt patients and in patients with post-nasal carcinoma. This discrepancy is likely due to the presence of different amounts of virus in antigen batches and in the increased sensitivity of our tests here, in which we develop the precipitating line with cadmium buffer. In one trial, Old obtained results identical to ours using serums sent to him from NCI and his antigen. The 133 human serums and the four antigens used in the Comparative Serology Survey of the Immunology Subgroup of the SVLP have been tested in this manner.

Antiserum prepared in monkeys against P-3 virus reacts with virus in a reaction of identity with the Burkitt patient's serum. The serum from a monkey inoculated with human "C-type" particles from an acute myeloid leukemia case also gave the same reaction of identity with the Burkitt patient's serum. Pre-bleedings from the monkeys did not react. This emphasizes a cross reaction between "C-type" and herpes-like particles.

When virus derived from various cell lines was tested, several were found to be cross reactive with or identical to the virus from P-3.

C. Neoplasia of Other Species -- Materials submitted by a variety of investigators were tested by immunodiffusion for cross reactivity with either murine viruses or Burkitt's derived herpes virus. These included extracts of kidneys of mice submitted by Dr. Lewis; tissue culture extracted virus submitted by Dr. Mitchell; and leukemic materials from cats submitted by Dr. Burger.

Interesting results have been obtained in tests from frogs bearing the Lucké tumor. In collaboration with both Dr. Mizell and Dr. King serums from frogs bearing the tumor have been tested with P-3 herpes virus antigen. A reaction of cross reactivity or identity between an antibody in the serum of some of the frog and in Burkitt patient's serum was found. This observation is preliminary and definitive interpretation awaits the availability of other frog preparations.

The serum from a monkey with radiation-induced leukemia, Monkey L-12, may have an antibody against one of the antigens of Rauscher virus. Similarly, the serum from a monkey inoculated in 1964 with the bone marrow of an acute erythroleukemia patient, as well as the serum from two monkeys used in serial passage of bone marrow, apparently contain an antibody reactive with Rauscher virus. These findings are preliminary, and definitive interpretation awaits testing of all available serum samples from these and other monkeys, as well as other selected control tests.

D. Mammary Tumor Virus -- Antiserum to the mammary tumor virus prepared in the rabbit, the Rhesus monkey, the C_3H agent-free mouse, and the BALB/c mouse has been tested for specific antibody by several techniques. The micro-immunodiffusion technique was used to show a line or lines of identity between antibody in each species against gradient purified, concentrated virus. In addition, under contract (PH43-66-458) neutralization tests have been performed using each of these serums; each showed a high ability to neutralize the mammary tumor virus. It has also been shown (see Sibal's report NCI-4824) that some of these serums are capable of hemagglutinating antigen-coated erythrocytes.

Studies of Roles of Humoral and Cellular Antibody in Immunity: A major goal of this laboratory is to describe the immune status of the natural host to a murine leukemia virus. Important to this is a consideration of the relative roles of cell-associated and humoral antibodies, to include an assessment of the extent that each type acts as either an anti-viral or anti-malignant cell inhibitor.

Past attempts to demonstrate delayed-type hypersensitivity (dependent on cell-associated antibody) led to only suggestive evidence that it occurred in murine leukemia. Techniques for the passive transfer of immunity by spleen cells from immunized mice have now been developed. Technical details for the methodology were worked out, principally by Dr. Cohen, as a preliminary to definitive experiments which were planned before his coming. In early experiments, the following observations were made:

1. Immune spleen cells inhibited the growth of Rauscher virus transformed malignant ascites cells. Lethal x-irradiation or freezing and thawing of these "immunocytes" eliminated their immunological competence.

2. Immune serum was also effective against the malignant ascites cell. Its effectiveness was removed by heat inactivation and restored by the addition of complement. Immune serum did not confer immunological capability upon normal spleen cells, nor did inactivated immune serum competitively inhibit the action of "immunocytes" on target cells.

A detailed experiment is now underway to test the effect of methods of immunization--including such variables as live versus killed virus vaccine, timing of booster inoculation, type of adjuvant used, etc.--on the following:

1. Reaction to challenge with malignant cells.

2. Reaction to challenge with virus.

3. Development of humoral antibody as measured by:
    a. immunodiffusion,
    b. passive hemagglutination,
    c. neutralization, and
    d. ability to reduce the transplantability of malignant cells when mixed with these cells in the presence of complement.

4. Development of cell-associated antibody as measured by the ability of spleen cells to reduce the transplantability of malignant cells when mixed with them prior to inoculation into recipient host.

Results of this experiment should give a firm basis for the understanding of the roles of cell-associated and humoral antibody in the hosts' defense against leukemia induced either by virus or by malignant cells.

Miscellaneous Experiments:

1. Two experiments have now been completed to determine whether BALB/c mice which are infected with a very low dose of Rauscher leukemia virus can be vaccinated with killed virus vaccine after infection. The preliminary experiment indicated that as measured both by the median spleen weight (0.9 g, control 1.7 g) and the median day to death (295, control 213) the vaccinated animals were partially protected against infection, even though all had succumbed by 810 days. Using a one log lower infectious dose, the second experiment has yielded the following results:

Immunizing Agent	No. Inoculated	% Dead at 637 Days	% Dead or Infected* at 637 Days
Vaccine & Adjuvant	20	5	5
Adjuvant & Saline	20	40	40
Nothing	20	25	50

* Gross disease apparent on autopsy after sacrifice at 637th day. Microscopic examination to be done.

These experiments indicate the feasibility of immunizing animals already infected with virus in an amount less than that necessary to produce overt disease, and argue in favor of the concept of a human leukemia vaccine even in the face of possible low grade infection of the majority of individuals.

Two similar experiments, (a) attempts to immunize AKR mice against spontaneous leukemia; and (b) attempts to immunize against radiation-induced leukemia prior to irradiation, originally planned for in-house, are to be performed under contract.

2. In recent years, an immunological adjuvant similar to Freund's adjuvant, but superior in that it contains only metabolizable ingredients, has been introduced by Hilleman as "adjuvant 65". While Freund's adjuvant has not been certified for human use because of untoward reactions, there is a possibility that adjuvant 65 might be. For this reason, the effectiveness of adjuvant 65 as compared to complete and incomplete Freund's adjuvant, as measured by the formation of humoral antibodies to Rauscher murine leukemia virus, was determined. As measured by micro-immunodiffusion, adjuvant 65 was as effective as complete Freund's, but by passive hemagglutination and neutralization, it was comparable to the less effective incomplete Freund's. Using this as a pilot experiment, more extensive studies will be performed under contract.

Major Findings:

1. Results of tests applying the immunodiffusion technique to the detection of antibody in human serums to the Burkitt herpes virus, show a high incidence of reactivity in serums from either normal or leukemia-lymphoma groups.

   Cross reactions or reactions of identity among herpes viruses isolated from several cell lines were demonstrated by immunodiffusion.

   A Rhesus antiserum against "C-type" particles from an acute myeloid leukemia, one against a herpes virus from Burkitt lymphoma, and serum from a Burkitt patient all show a reaction of identity with Burkitt herpes virus from the P-3 cell line.

2. In two experiments, terminated 810 and 637 days, it was shown that BALB/c mice infected prior to vaccination with Rauscher virus are less susceptible to leukemia as measured by reduction in median spleen weight, an increase in the median time to death, and a significantly lower incidence of leukemia.

3. Preliminary studies on the nucleoid antigen of eight or more strains of murine leukemia virus indicate that most, if not all, of the strains share an antigen which is either identical or strongly cross reactive.

4. Methods developed in this laboratory, including immunodiffusion and passive hemagglutination inhibition, revealed that a virus recovered from a mouse inoculated with human leukemic materials was of murine origin. The applicability of this type of testing to the species identification of viruses isolated under similar circumstances in other situations is stressed.

5. Mammary tumor virus antiserums prepared in the Rhesus monkey, the rabbit, C_3H mice, and BALB/c mice have antibody detectable by immunodiffusion, by neutralization, and by passive hemagglutination.

Significance to Biomedical Research and the Program of the Institute:

The entire research approach of this laboratory is goal oriented, being concerned almost entirely with the solution of problems of diagnosis, therapy, and prevention of human leukemia. In order to solve these problems, however, it is necessary to work with animal systems which are amenable to manipulation in the laboratory, and to apply the basic findings made in these models to the human system. The significance to biomedical research and the program of the Institute is thus viewed as being twofold:

1. Basic scientific knowledge regarding the immunological response to leukemia has been acquired using a murine virus model system. This basic knowledge has been and will continue to be applied to studies of the diagnosis, treatment, or prevention of the disease in the natural host.

2. The Special Virus-Leukemia Program of the National Cancer Institute has as its goal the development of methods for control of human leukemia. This program is directly served by the application of findings made in murine leukemia to the human problem. At this time two techniques--immunofluorescence and immunodiffusion--first used in the model system, have been exploited in the human disease. It is anticipated that models for immunotherapy in man will be forthcoming.

Proposed Course of Project:

1. Inherent in the understanding of the disease is the need to assess the roles of the different kinds of specific immunity which arise as a result of infection or vaccination with leukemia virus antigens. Inasmuch as there is now strong evidence for a protective role for both humoral and cellular antibodies, we propose within the coming year to administer a combination of humoral and cell antibody to leukemic mice in an attempt at therapy.

2. Another therapeutic trial which will be attempted is that suggested by the work of Dr. Peter Alexander at the Chester Beatty Institute in London. Heterologous species will be immunized with a murine leukemia virus. Lymph draining the site of inoculation will be examined for the appearance of an RNA-rich "immunocyte", and at the time when this cell is most abundant the lymph will be obtained by cannulation of the duct, the RNA extracted, and attempts made to treat leukemic animals with this extract.

3. Preliminary work has revealed an antigen of the nucleoid of the virus which is easily separable and which apparently is present in most of the murine leukemia species. We are unable, however, to confirm Dr. O'Connor's observation of the separation of antigens of the outer coat in the ether phase, and we expect to pursue this problem using other approaches. Most fortunately a biochemist will report for duty in the Section in July, and will pursue this area of research.

4. One major effort planned for the past three years but awaiting the necessary materials and space was the cross immunization study using at least eight different strains of murine leukemia virus. The materials have been delivered from the contractor, and the

entire study is to begin this year under a contract to NCI using protocols devised at NCI. This work should lead to the selection of strains necessary in making a multivalent vaccine against murine leukemia.

5. In July 1968 the experimental work of the Comparative Serology Survey begun by the Immunology Subgroup of the SVLP will be complete. After data are analyzed in Dr. Waggoner's office, the most appropriate test or tests for the detection of antibody to Burkitt herpes virus will be known, and an intensive sero-epidemiologic survey can be quickly mounted whenever such a study is judged to be desirable.

   A main interest of the Immunology Subgroup, and an increased effort of the Immunology Section, will turn by personal interest and at the request of the Associate Scientific Director, to a concerted investigation of immunological cross reactivity among suspected leukemia-inducing agents from man and lower animals.

Publications
(other than abstracts from this project)

Fink, Mary A. and Sibal, Louis R.: Comments on "Virus Specific Antigens in Viral Tumors". In Trentin, J. J. (Ed.): Cross-Reacting Antigens and Neoantigens. Baltimore, Md., Williams & Wilkins, 1967, pp. 78-84.

Fink, Mary Alexander, Herbut, Peter A., Sibal, Louis R., and Wivel, Nelson A.: Probable Murine Origin of a Virus (HHLV-2) Recovered from Leukemic Mice after Inoculation of Human Leukemia Material. JNCI, 39: No. 6, 1253-1255, 1967.

Fink, Mary A.: Studies of anti-"C-type Particle" Fluorescent Antibody in Human Leukemia: A Status Report. In Proceedings of the Third International Symposium on Comparative Leukemia Research. Basel, Switzerland, S. Karger, 1968, Volume 6, No. 8 (In Press).

Sibal, Louis R., Fink, Mary Alexander, Robertson, Diane D., and Cowles, Carolyn A.: Enhanced Production of Antibody to a Murine Leukemia Virus (Rauscher) in the Host of Origin. Proc. Soc. Exp. Bio. Med., 127: 43-47, 1968.

Fink, Mary Alexander and Cowles, Carolyn A.: Use of Immunological Techniques in the Study of Human Leukemia. In Zarafonetis, C. J. D. (Ed.): Proceedings of the International Conference on Leukemia/Lymphoma. Philadelphia, Penna., Lea & Febiger, 1968 (In Press).

Sibal, Louis R., Fink, Mary Alexander, and Robertson, Diane D.: Quantitative Measurement of a Murine Leukemia Virus (Rauscher). (Accepted after revision for publication in Virology.)

Serial No. NCI-4824
1. Viral Leukemia & Lymphoma Branch
OASDVO, Etiology
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: The development of immunological systems for the study of tumor viruses to include methods for the measurement of antibodies, for the quantitation of virus, and for the detection of antigens of virus as related to the diagnosis of viral neoplasia.

Principal Investigator: Dr. Louis R. Sibal

Other Investigators: Dr. Mary A. Fink
Dr. Max H. Cohen

Cooperating Units: Inside NIH:

Laboratory Aids Unit
Dr. Nelson Wivel
Dr. John Moloney
Dr. Timothy O'Connor
Dr. Janet Hartley (NIAID)

Man Years: (July 1, 1967 through June 30, 1968)

Total: 4.6
Professional: 1.1; non-research 0.15 (SVLP administrative and contractual work)

Others: 3.35

Project Description

Objectives:

The basic aims of this project are to develop qualitative and quantitative methods (a) for the detection of viruses and (b) for antibodies to those viruses as related to neoplasia.

The principal model system used in these studies has been the murine leukemia viruses, mainly the virus strain isolated by Rauscher. Studies have also been conducted with a strain of herpes-type virus isolated from

Burkitt's lymphoma. In general, findings made in the murine model system are exploited in the human counterpart to determine their applicability to the diagnosis of viral neoplasia in man.

Methods Employed:

Serological Detection of Antibody and Virus:

A. Murine Leukemia:

1. Passive hemagglutination (HA) tests -- This test, in which tanned SRBC coated with gradient purified virus are agglutinated in the presence of virus-specific antibodies is now performed routinely. This technique has become the principal method for titrating antibodies in animal serums. Basic procedures described originally in the 1967 report have remained unchanged.

   Glutaraldehyde treatment of SRBC has supplanted formalin treatment as the method of choice for preparing sensitized cells suitable for HA tests. The virus coated cells are stable for months at 4° C. This availability of standard preparations of sensitized SRBC makes this test especially valuable in sero-epidemiologic studies.

   Attempts to improve the HA test by using coupling agents other than tannic acid were unsuccessful.

2. Hemagglutination-inhibition (HAI) tests -- Preliminary studies indicated that the HA reaction could be quantitatively inhibited by virus, thus providing a rapid, *in vitro* method for measuring virus. A standard assay system has now been devised and many different preparations containing virus have been titrated. Concentrated tissue culture supernatant fluids (PH43-66-98), plasma and organ extracts from infected mice (PH43-66-98 and PH43-64-533) and various fractions from density gradient ultracentrifugation (PH43-66-98) may be directly examined for their content of viral antigen since corresponding control preparations do not show inhibitory activity. Studies have shown good correlation between HAI activity and electron microscopic particle counts (PH43-66-98). Significantly, the high degree of correlation between HAI activity and bioassays (PH43-67-697) indicate that when virus is prepared and handled by a standard protocol, HAI titers accurately reflect the amounts of virus present. This assay system can be used to screen standard virus preparations as a preliminary to or in place of laborious infectivity assays.

   Under direct supervision of this laboratory, this test is now performed at Microbiological Associates, Walkersville (PH43-67-697).

3. Cytotoxicity tests -- The radiochromium cytotoxicity assay has been adapted for use in the murine leukemia system. Preliminary experiments performed by Dr. Cohen have shown that this test is specific for the assay of anti-viral antibodies in serums of Rhesus monkeys and BALB/c mice immunized with Rauscher virus vaccine. The sensitivity of this technique is being compared to other serological tests currently in use by this section.

4. Localized hemolysis-in-gel tests -- The Jerne technique, which makes possible the study of the cytodynamics of the immune response is now performed in this laboratory. The method is being tested for its applicability to the study of antibody formation of mice to murine leukemia viruses. Conditions for a successful experiment have not yet been defined.

B. Human Leukemia: We have attempted to develop the passive HA technique for the detection of antibodies to the herpes-type virus isolated from a Burkitt's lymphoma cell line (P-3) primarily because the method is sensitive and can be readily adapted to seroepidemiologic surveys. Enzyme-treated, gradient purified P-3 virus was used as the antigen for the sensitization of tanned SRBC (PH43-66-98). Although antiserum from Rhesus monkeys immunized with P-3 virus reacted strongly in HA tests, we have been unable to demonstrate that the reaction is virus specific. Burkitt's lymphoma patients' serums rarely reacted in HA tests.

This laboratory has participated in a cooperative program organized by the SVLP Immunology Subgroup to test human serums for antibodies to the herpes-type virus. (See Dr. Fink's report (NCI-4823) for further information.) Using the passive HA technique, 133 serums have been tested against four antigens provided by this group. The results will be reported to Dr. Waggoner's office before July 1, 1968; however, in the light of our present findings, it may be necessary to withdraw from testing before that time.

Murine Virus Studies: Plasma and spleen extracts of the following virus strains showed cross reactivity with Rauscher virus in HAI tests: Moloney, Friend, Kirsten, Gross, Manaker C-60, Breyere-Moloney, Rich; Buffett virus was not reactive. These results confirm those of Dr. Fink using the micro-immunodiffusion method. See report of Dr. Fink's (NCI-4823) for further information.

A virus isolated from Swiss mice inoculated with human leukemia material showed strong cross reactivity with Rauscher virus in HAI tests. See report of Dr. Fink's (NCI-4923) for further information.

Evidence from HAI studies indicated that ficin-treatment of tissue culture derived Rauscher virus removes an antigen (PH43-66-98). Absorption of antiserum with enzyme-treated virus completely removed reactivity to this virus; reactivity to untreated virus was unaffected. Absorption with untreated virus removed reactivity to both virus preparations. The findings confirm those obtained earlier by micro-immunodiffusion (Fink). The identification of this antigen is necessary to assess the significance of this observation.

Antigens extracted from the nucleoid of the virion of Rauscher murine leukemia react strongly in HAI test. Comparison with the complement-fixation reaction by Dr. Hartley indicates that HAI is significantly more sensitive, i.e., 1:80 by HAI and 1:8 by CF. Further studies on tween-ether treated virus are in progress.

Serums of BALB/c mice inoculated with Rauscher virus vaccine plus endotoxin showed HA activity which was detected as early as the 3rd day following primary stimulation. Peak titers were reached by the 7th day. In contrast, mice given vaccine alone failed to respond. HA activity was restricted to the gamma-M globulin fractions. Representative serums, all of which showed measurable HA activity, failed to precipitate or neutralize virus.

Major Findings:

1. HAI tests provide a rapid, in vitro method for quantitative assay of a murine leukemia virus antigen. Such tests are significantly more sensitive than the complement-fixation method suggested by Hartley et al.

2. Endotoxin markedly enhances the early gamma-M primary response of the syngeneic host to a murine leukemia virus.

3. Viral antigen from many strains of murine leukemia viruses cross reacts in the Rauscher HAI system.

Significance to Biomedical Research and the Program of the Institute:

The development of sensitive serological tests for the detection of antibodies to a leukemia virus and for the detection of virion antigens contribute to the elucidation of the antigenic structure of individual viruses and possible antigenic inter-relationships among the viruses. Such information is significant to biomedical research. Application of these findings supports the aim of control of leukemia in the host. While it has been necessary to work with animal systems which are amenable to laboratory investigation, the ultimate goal of this project is to aid in the solution of problems of diagnosis of human leukemia.

Proposed Course of Project:

Efforts of this laboratory have been directed toward developing workable solutions to the problems associated with viral lymphoma and leukemia. Any significant findings in the area of murine leukemia are then applied, if feasible, to those in human leukemia.

Specific plans for the coming year:

Collaborative studies on the elucidation of the antigenic structure of a murine leukemia virus will be continued.

Studies on the serological and protective activities of early (gamma-M) and late (gamma-G) antibodies will be continued.

A major effort of this laboratory will be to develop the passive HA test into a sensitive, specific method for the detection of antibodies to the herpes-type particle isolated from Burkitt's lymphoma cell lines (P-3). Experiments to increase the potency or reactivity of these viral antigens are already underway.

Attempts to apply the Jerne plaque test for studying the antibody response at the cellular level will be continued.

Recent evidence has indicated that renal glomeruli may be a source of antibodies in chronic viral infections eliciting a low antibody response. Efforts will be made to determine whether or not antibody to a murine leukemia virus have been deposited in the mouse kidney in the form of complexes of antibody and virus.

Publications
(other than abstracts from this project)

Fink, Mary A. and Sibal, Louis R.: Comments on "Virus Specific Antigens in Viral Tumors". In Trentin, J. J. (Ed.): Cross-Reacting Antigens and Neoantigens. Baltimore, Md., Williams & Wilkins, 1967, pp. 78-84.

Fink, Mary A., Herbut, Peter A., Sibal, Louis R., and Wivel, Nelson A.: Probable Murine Origin of a Virus (HHLV-2) Recovered from Leukemia Mice after Inoculation of Human Leukemia Material. JNCI 39: 6, 1253-1255, 1967.

Sibal, Louis R., Fink, Mary A., Robertson, Diane D., and Cowles, Carolyn A.: Enhanced Production of Antibody to a Murine Leukemia Virus (Rauscher) in the Host of Origin. Proc. Soc. Exp. Bio. Med. 127: 43-47, 1968.

Sibal, Louis R., Fink, Mary A., and Robertson, Diane D.: Quantitative Measurement of a Murine Leukemia Virus (Rauscher). (Accepted after revision for publication in Virology.)

Serial No. NCI-4825
1. Viral Leukemia & Lymphoma Branch
OASDVO, Etiology
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Use of antilymphocyte serum as an immunosuppressant in the Rhesus monkey.

Principal Investigator: Dr. Max H. Cohen

Other Investigators: Dr. Mary A. Fink

Man Years: (July 1, 1967 through June 30, 1968)

Total: 0.25

Professional: 0.25

Project Description

Objectives:

This program is being carried out in conjunction with the continuing effort by the Institute to transplant or transmit human leukemia or lymphoma to sub-human primates. The objective of the current project is to determine whether antiserum prepared against monkey lymphoid cells, alone or in combination with other agents, can be utilized in the Rhesus monkey as a potent immunosuppressant of relatively low toxicity.

Methods Employed:

Antiserum has been prepared in rabbits by intraperitoneal injection of monkey thymus tissue culture cells (PH43-67-661) emulsified in Freund's adjuvant. This was followed by subcutaneous booster injections of the cells without adjuvant. Animals were subsequently bled at weekly intervals and the serum stored at -20° C until administered to the monkeys.

The initial two newborn monkeys have been inoculated subcutaneously and intramuscularly with Rauscher virus-transformed malignant ascites cells. These newborn animals, one of which is being treated with ALS, the other with normal rabbit serum, are being followed for the development of local tumors, systemic illness, and for an alteration of humoral response to injected material. Their serums will be tested by micro-immunodiffusion for reaction with Rauscher virus, rabbit serum, and mouse plasma.

Major Findings:

Preliminary evidence indicates that antilymphocytic serum produced in the rabbit by the above method is effective in producing in the monkey a depression of the white blood cell count and of the absolute lymphocyte count. This antiserum also produces a precipitin reaction against monkey serum when tested by the Ouchterlony technique.

No data are yet available concerning the effect of the ALS on the monkey's responses to the injected materials.

Significance to Biomedical Research and the Program of the Institute:

The need to transmit human leukemia to a lower species is of paramount importance. Antilymphocyte serum has been used successfully to facilitate grafting of normal tissues, and transplantation of malignant tumors from one species to another. It is anticipated that ALS may enhance the growth of human tumors in the primate. This project thus serves a major goal of the Special Virus-Leukemia Program.

Proposed Course of Project:

ALS-suppressed primates will be challenged initially with murine leukemia virus and transformed cells in order to develop an experimental basis for the later injection of test human materials, such as human lymphomatous material and virus preparations of unknown oncogenic potential.

Serial No. NCI - 4818
1. Viral Leukemia & Lymphoma Branch,
   OASDVO, Etiology
2. Molecular Virology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Biophysical Studies on Animal Leukemia Viruses

Previous Serial Number: Same

Principal Investigator: Timothy E. O'Connor

Other Investigators: Dr. Peter Fischinger
Dr. John Fogelman
Dr. Shalom Hirschman
Mrs. Johanne Messore
Mrs. Carolyn Moore

Cooperating Units: None

Man Years
Total: 4
Professional: 2
Other: 2

Project Description

Objectives:

The development of an interrelated and comprehensive knowledge of the biophysics, biochemistry and cellular biology of animal leukemia viruses with a view to its employment in the demonstration of the possible association of a virus with human leukemia.

Methods Employed:

The interaction of murine sarcoma-leukemia virus complex with murine cells in tissue culture was extensively examined to elucidate the quantitative cellular biology of these viral agents. Focus assay of the murine sarcoma virus in mouse embryo tissue cultures was an essential quantitative tool in these studies. Biophysical characterization of the viral agents (for example, density gradient centrifugation) was employed where appropriate.

Major Findings:

(1) Study of the focus assay titration patterns of various stocks of the Moloney sarcoma virus (MSV) confirmed the earlier finding of the defectiveness of MSV. However, the titration patterns of various stocks were quite variable and the individual titration patterns were complex and not consistent with either "one-hit" or "two-hit" kinetics of cellular infection. These anomalies were rationalized by the postulate that the virus stocks contained variable but significant amounts of competent sarcoma virion, which did not require the involvement of a leukemia virion for focus production. The available data indicated that the competent sarcoma virion probably consisted of zygotic hybrid of a sarcoma virion and a leukemia virion. Hybrid RNA tumor viruses have previously not been described.

(2) The elucidation of the composition of stocks of murine sarcoma virus in terms of a three-fold mixture of defective sarcoma virus, competent sarcoma virus, and excess leukemia virus led to the formulation of the quantitative effect of any exogenous leukemia virus stock on an indicator MSV stock of known composition. This led to the development of a rapid assay system for murine leukemia viruses in tissue culture based on the helper activity for focus production by indicator MSV stocks. This assay system has several advantages: a) only simple tissue culture technology and MSV focus assay procedures on mouse embryo fibroblasts are employed, b) the 6 day titration of the test is unprecedented in rapidity, c) the sensitivity and reproducibility are excellent over a wide range of helper activity. All of the strains of murine leukemia virus examined to date have been shown to be active whether derived directly from infected animals or from tissue culture passages of the respective viruses. The assay should prove helpful in the elucidation of the molecular events following infection of mouse cells with leukemia viruses.

(3) Any stock of MSV of defined composition can be employed in the leukemia virus helper assay, but virus stocks having a high content of defective sarcoma virion have the greatest sensitivity. The early viral harvests which are obtained following infection of the 3T3 mouse cell line with competent sarcoma virus are particularly useful in this assay since they contain a high level of defective sarcoma virion.

(4) The Harvey strain of the murine sarcoma virus shows behavior similar to the Moloney strain on infection of mouse embryo cultures. Preliminary experiments indicate that highly defective stocks of this agent, which are useful in the helper assay of leukemia virus, can be obtained by passage of the agent in rat embryo tissue cultures. These stocks give highly defective titration patterns which can be normalized by addition of murine leukemia virus when plated on mouse embryo fibroblasts.

(5) Study of the infection of various mouse cell systems with murine leukemia virus using the helper assay to follow growth of progeny virus has revealed that tissue cultures derived from various organs of the same mouse strain can

differ widely in their ability to support leukemia virus propagation. Thus, mouse kidney cultures are relatively poor supporters of leukemia virus propagation as compared to mouse embryo cultures.

(6) The availability of both the focus assay of the sarcoma virus and the helper assay of the leukemia virus has permitted an examination of the propagation of each of the components of the sarcoma-leukemia virus complex in various mouse cell cultures. It is particularly noteworthy that some cell cultures (for example, mouse kidney cultures) which have a low capacity to support leukemia virus propagation readily support propagation of competent sarcoma virion. On the other hand mixtures of defective sarcoma virus and leukemia virus grow poorly in such cell systems. This clearly indicates the biological advantage enjoyed by the competent sarcoma virus, which probably consists of a sarcoma-leukemia virus hybrid.

The composition of stocks of the sarcoma-leukemia virus complex can be altered by passage through cells having a preferential capacity to support replication of one of the components of the viral complex. This phenomenon of alteration of composition by passage in appropriate cells we have described as Modulation due to its familiar analogy with other physical processes in which one signal is selected from several input signals. Modulation appears to be an important phenomena in infection of cells with multi-component virus mixtures. Further elucidations of this phenomenon should contribute to our understanding of disease induction by multi-component virus mixtures.

Significance to Bio-medical Research and the Program of the Institute:

During the period of the present report the elucidation of the existance of competent (hybrid) sarcoma virions, the development of a helper assay for leukemia virus and the elucidation of the phenomenon of Modulation have provided new insights into the cellular biology of the sarcoma-leukemia virus complex. It is planned to further elucidate the phenomena involved in these processes. The new technology which should hopefully result from this investigation should provide a sound basis for attempts at isolation of human sarcoma or leukemia viruses.

Proposed Course:

As the quantitative biology of the above processes is defined the underlying molecular mechanisms will be more extensively explored.

Honors or Awards:

Dr. T. O'Connor was a member of the delegation of U.S. virologists that visited the Soviet Union in September, 1967 under the terms of a U.S.-Soviet Health Exchange. As Rapporteur of the delegation, Dr. O'Connor wrote the official report. He presented a seminar at the Gamaleya Institute For Epidemiology and

Microbiology, Moscow on September 21, 1967 entitled, "*In Vitro* Studies on the Defectiveness of the Murine Sarcoma Virus." Dr. O'Connor also spoke on the same topic at the Kirchenstein Institute at Riga on September 26, 1967.

Dr. O'Connor presented by invitation a talk on "Recent Research Findings on the Defectiveness of the Murine Sarcoma Virus" at the Statens Veterinarmedicinska Anstalt, Stockholm on October 4, 1967.

On October 11, 1967 he presented a seminar on "Recent Research Findings on the Murine Sarcoma-Leukemia Virus Complex" at the Max-Planck Institute fur Virusforschung in Tubingen, Germany.

Dr. O'Connor presented a seminar in Camden, New Jersey on October 26, 1967 at the Medical Research Foundation entitled, "Studies on Defective Viruses".

Dr. O'Connor presented a seminar entitled, "Studies on a Defective Virus and Their Possible Application to Human Solid Tumors" at the M.D. Anderson Hospital in Houston, Texas on February 22, 1968.

Dr. O'Connor presented a talk entitled, "Some Aspects of the Defectiveness of the Moloney Sarcoma Virus," at the Information Meeting of the Special Virus Leukemia Program at the National Institutes of Health on February 28, 1968.

Dr. O'Connor has been selected to present a paper entitled, "Helper Assay of Murine Leukemia Viruses" at the Tissue Culture Association Meeting in San Juan, Puerto Rico on June 10, 1968.

Publications:

O'Connor, T.E.: Quantitative *in vitro* studies on a murine sarcoma-leukemia virus complex. *Perspectives in Leukemia.* New York, New York, Grune and Stratton, 1968, pp. 64-80.

O'Connor, T.E. and Fischinger, P.J.: Titration patterns of a murine sarcoma-leukemia virus complex: Evidence for existence of competent sarcoma virions. *Science*, 159: 325-329, Jan. 1968.

Monroe, J., Shibley, G., Schidlovsky, G., Nakai, T., Howatson, A., Wivel, N., and O'Connor, T.: Action of snake venom on Rauscher virus. *J. Nat. Cancer Inst.* 40: 135-145, Jan. 1968.

O'Connor, T.E. and Fischinger, P.J.: Tissue culture assay of helper activity of murine leukemia virus for murine sarcoma virus. *J. Nat. Cancer Inst.* 1968 (In Press).

O'Connor, T.E.: U.S.-Soviet Health Exchange in Virology, 1967. *Science* (In Press).

Serial No. NCI - 4819
1. Viral Leukemia & Lymphoma Branch,
   OASDVO, Etiology
2. Molecular Virology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Moloney Sarcoma Virus: Interactions with Leukemia Viruses in Murine Cell Systems

Previous Serial Number: Same

Principal Investigator: Peter J. Fischinger

Other Investigators: Timothy E. O'Connor
Mrs. Carolyn Moore

Cooperating Units: None

Man Years

Total:	2.0
Professional:	2.0
Others:	0

Project Description

Objectives:

The primary objective is the understanding of Moloney sarcoma virus-cell interactions which lead to cellular transformation. The nature of virus titration patterns which indicate a varying degree of viral defectiveness and competence has to be clarified. Intrinsic cellular control mechanisms which dictate the susceptibility of a cell system to MSV infection have to be delineated to understand intra and trans-species cellular neoplasia.

Secondary objectives include the development of a rapid quantitative tissue culture assay for murine leukemia viruses (LV). Eventually the biological cell-virus interactions are to be related to subviral and macromolecular levels.

Methods Employed:

Standard methods of cellular biology and virology.

Major Findings:

The examination of titration patterns of MSV from several sources revealed varying slopes ranging from one-to two-hit kinetics. Proposed model titrations of MSV both with and without added LV were compared with actual tissue culture infections. Both suggested the existence of a competent MSV which did not need a LV for replication or focus formation. The genetic instability of competent MSV, its similarity of coat to MLV, and a failure to detect sufficient LV beyond the sarcoma endpoint suggested that the competent virion is a hybrid of MSV and LV.

A simple, rapid, tissue culture assay for murine LV was realized. Leukemia virus could be quantitatively expressed in "leukemia virus helper units" (LVHU) as helper activity for defective MSV in mouse embryo tissue culture. Model relationships verified the universal feasibility of any defective or partially defective MSV stock to act as an indicator for LV quantitation.

Cellular control of MSV-LV complex was found to be of great importance. Depending on the murine cell system used, MSV could effect repeated transitions between defective and competent states. This change was termed "modulation". Growth curves of either defective MSV and LV, or competent MSV were performed in MEF, 3T3 and neonatal mouse kidney cells. The efficiency of infection in MEF cells was similar for either defective or competent forms of MSV. In contrast 3T3 and kidney cells were relatively nonpermissive for defective MSV. Pure LV growth was also assessed in these three cell types. The timing and titer of progeny LV was found to correspond closely to the rate of growth exhibited by defective MSV in each cell type. Competent MSV could overcome the cellular insusceptibility and replicate rapidly in 3T3 and neonatal kidney cells, further suggesting that the competent particle is a hybrid.

Chronic cellular infection of MEF or 3T3 cells with MSV established a steady state equilibrium relative to the quantity of LV, defective MSV and competent MSV produced on subsequent passage levels. Chronic infection of MEF and 3T3 cells with RLV was easily obtained. Because the nature of the LV helper in MSV stocks was never definitively determined, 3T3 cells were infected with terminal dilutions of MSV. Some of the cultures obtained from single clones were insusceptible to MSV and produced a helper virus. Subsequent mouse inoculations with helper infected 3T3 cell lysates initiated a typical Moloney leukemia syndrome. Cell free organ extracts of these leukemic mice rapidly induced Moloney leukemia in new recipients.

Significance to Bio-medical Research and the Program of the Institute:

The postulate of a potential LV-MSV genetic composite constitutes the first description of an RNA tumor virus hybrid.

The tissue culture assay for LV as helper for the defective MSV represents a simple, rapid, and sensitive assay for which neither special reagents nor specialized technology are required.

The demonstration that a whole tumor virus stock can modulate between defectiveness and competence is a new phenomenon with profound implications for tumor virology and carcinogenesis.

Proposed Course:

The immediate goals are the determination of the nature of the MSV-LV hybrid at the molecular level. Viral modulation is to be extended to other murine cell types. Cellular contribution to viral modulation should hopefully lead to a facilitation to crossing of species barriers. The nature of helper activity is under close scrutiny. Eventually the understanding of these basic reactions in the murine system should elicit meaningful and profitable approaches to the determination of the role of viruses in human neoplasia.

Honors and Awards:

Dr. Fischinger presented a seminar on "Defective Viruses" to the Pathology Branch, National Cancer Institute on 2/9/68.

Dr. Fischinger presented a seminar entitled, "Modulation of a Sarcoma-Leukemia Virus Complex in Cell Systems" to the Molecular Biology Study Group, National Institutes of Health on 4/12/68.

Dr. Fischinger presented a paper entitled,"Modulation of Defective and Competent Forms of MSV in Murine Tissue Cultures" at the Meeting of the Tissue Culture Association at San Juan, Puerto Rico, 6/12/68.

Publications:

O'Connor, T.E. and Fischinger, P.J.: Titration patterns of a murine sarcoma-leukemia virus complex: Evidence for existence of competent sarcoma virions. Science 159: 325-329, Jan. 1968.

Fischinger, P.J. and O'Connor, T.E.: Tissue culture assay of helper activity of murine leukemia virus for murine sarcoma virus. J. Nat. Cancer Inst. In Press.

Serial No. NCI - 4820
1. Viral Leukemia & Lymphoma Branch, OASDVO, Etiology
2. Molecular Virology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Dose Dependence and Virus Content of Tumors Induced in Mice With Murine Sarcoma Virus (Moloney)

Previous Serial Number: Same

Principal Investigator: John A. Fogelman

Other Investigators: Timothy E. O'Connor

Cooperating Units: None

Man Years
Total: 2
Professional: 1
Other: 1

Project Description

Objectives:

The work is directed towards the description of the dose-response relation of the intact murine host to a murine sarcoma virus (Moloney), and to evaluation of the virus content of the tumors thus produced. Special consideration is focused upon the influence of the sarcoma virion composition of the virus stocks, particularly the relative degree of defectiveness/competence. Special efforts are directed towards the production of "non-infective" tumors (i.e. non-virus producing). Evaluation of the non-infectivity of such tumors is made by attempting to demonstrate the recoverability of virus from them by *in vivo* and *in vitro* "rescue" techniques.

Methods Employed:

The methods employed are those familiar to workers with oncogenic viruses. Special advantage is taken of the quantitative aspects of the sarcoma virus focus assay performed with coinfection by leukemia virus helper. This technique is especially useful for the demonstration of recoverable virus from "non-infective" tumors.

Major Findings:

The results indicate that the host response in both BALB/c and NIH Swiss mouse as measured by percent tumor incidence was directly related to the initiating dose, and that the latent period was inversely related to the dose. The percent tumor incidence response did not depend upon the sarcoma virion composition, but the data suggest that the latent period was related to the degree of competence/defectiveness.

Of the 48 cell-free tumor extracts that were assayed for virus content, all but four were "infective", i.e. had focus-forming activity without coinfection by leukemia virus helper in the in vitro assay. The virus content of these "infective" tumors was not related to the virus initiating dose nor to the defective/competent sarcoma virion composition of the virus stock. Two of the four "noninfective" tumors had very low-titer recoverable virus on coinfecting in the focus assay with leukemia virus. Only two "non-infective" tumors had no demonstrable in vitro focus activity in the helper-assisted assay. However, one of these latter two cell-free tumor extracts produced tumors in the intact NIH Swiss host. The initiation of the "non-infective" tumors was not related to either virus initiating dose or the sarcoma virion composition.

Significance to Bio-medical Research and the Program of the Institute:

The entire focus of the study reported here is directed towards the goal of our Institute: to advance our understanding and management of the neoplastic process. Specifically, our objective is to exploit the techniques of viral recovery, to be developed in the animal models, so that they may be applied to human tumor material for the eventual demonstration of their viral origin.

Proposed Course:

This project will be completed near the end of the fiscal year.

Serial No. NCI - 4821
1. Viral Leukemia & Lymphoma Branch,
   OASDVO, Etiology
2. Molecular Virology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Study of Macromolecular Synthesis Occuring in Early Phase of MSV Infection in Mouse Cells in Tissue Culture

Previous Serial Number: None

Principal Investigator: Shalom Z. Hirschman

Other Investigators: Timothy E. O'Connor
Peter J. Fischinger

Cooperating Units: None

Man Years
Total: 2
Professional: 1
Other: 1

Project Description

Objectives:

The molecular events involved in the growth of MSV in tissue culture and in its interaction with helper leukemia viruses are being investigated. Antimetabolites with specific inhibitory effects on macromolecular synthesis are being utilized to study the biochemical events occurring following infection of mouse embryo fibroblasts and 3T3 cells in tissue culture with MSV and associated helper leukemia virus. The rates and total quantity of macromolecules synthesized in the early period following infection, are being followed by use of radioactive tracers. Our aim is to isolate the virus specific macromolecules synthesized during infection. These macromolecules will be biologically and physically characterized and their synthesis will be attempted in cell free systems with the use of viral and host cell enzymes. The deciphering of the biochemical events occurring during virus growth may allow for the development of methods for selective inhibition of viral growth without harming the host cell.

In the course of these studies data collection will be automated as much as possible and data calculation and retrieval will be facilitated by computer techniques.

Methods Employed:

The focus assay for MSV as refined by Drs. O'Connor and Fischinger is employed to follow growth of virus. The radioactive labeling techniques are standard and the methods for studying DNA are those defined by Drs. Hirschman and Felsenfeld.

Major Findings:

The requirement for DNA synthesis early in infection of mouse cells by MSV has been established. The time course for DNA synthesis occurring after virus absorption onto host cell has been charted. Antimetabolites which inhibit DNA synthesis do not interfere with viral absorption onto 3T3 host cells. Viral growth is more sensitive to DNA inhibition during early infection than is cellular growth. The quantitative relationships between concentration of antimetabolite and extent of inhibition of viral growth have been explored.

Significance to Biomedical Research and the Program of the Institute:

The understanding of the biochemical events attending oncogenic virus replication may aid in developing methods for control of human cancers. The finding that DNA replication is a prerequisite for growth of this RNA virus raises many important questions as to the mechanism of viral RNA and protein synthesis. From the aspect of basic knowledge this study is of utmost importance in giving insight into the interaction of mammalian cells and RNA core viruses.

Proposed Course:

This study, now in its early stages, will be continued and amplified. Efforts are underway to purify and concentrate both the leukemia and sarcoma viruses and to characterize their respective RNAs. Techniques are being developed for the isolation of the DNA being synthesized during early infection. This DNA will be carefully studied as to its base composition, intramolecular heterogeneity and homology with both leukemia and sarcoma virus.

Publications:

Hirschman, S.Z., Gellert, M., Falkow, S., and Felsenfeld, G.: Spectral analysis of the intramolecular hetergeneity of lambda DNA. J. Mol. Biol. 28: 469-477, 1967.

Hirschman, S.Z., Leng, M., and Felsenfeld, G.: Interaction of spermine and DNA. Biopolymers. 5: 227-233, 1967.

Serial No. NCI-4833
1. Viral Leukemia & Lymphoma Branch
OASDVO, Etiology
2. Ultrastructural Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Electron Microscope Studies of Normal, Malignant and Virus-Infected Cells and Cell Fractions.

Previous Serial Number: Same

Principal Investigator: Dr. Nelson A. Wivel

Other Investigators: None

Cooperating Units:
Dr. E. L. Kuff, Biochemistry Branch (NCI-366)
Dr. M. Potter, Biology Branch
Dr. Richard Asofsky
Dr. Mark Mandel
Dr. Mary A. Fink
Dr. L. R. Sibal
Dr. Padman Sarma
Dr. M. A. Chirigos

Man Years:

Total:	2.5
Professional:	1.0
Other:	1.5

Project Description

Objectives:

To utilize the techniques of electron microscopy to describe and elucidate the ultrastructure of malignant cells, and to define the relationships between the presence of viruses and the existence of neoplasia. To monitor and correlate the contributions from various disciplines such as biochemistry and immunology in an understanding of carcinogenesis and cellular differentiation.

Methods Employed:

The principal methods involve the visualization of appropriate selected biological materials in the electron microscope, utilizing techniques of thin sectioning and negative staining.

Major Findings:

1. Studies are continuing in an attempt to define the role of the intracisternal A-particle which occurs in murine plasma cell tumors. The techniques of isolation of these particles have been refined to produce large enough quantities for biochemical studies. Present efforts in collaboration with Dr. E. L. Kuff are directed toward identification and characterization of the proteins in the inner shell of the particles. Purified preparations of these particles are being tested serologically in order to determine the possible relationships to the intracytoplasmic A-particles which are seen in mouse mammary tumors. The results of the isolation studies have been submitted in manuscript form for publication.

2. Ultrastructural studies of the mineral oil-induced and plastic disc-induced mouse plasma cell tumors are continuing. Preliminary results indicate that A-particles may be present in various cell types in the peritoneal cavity prior to the overt development of tumors. Several of the established plasma cell tumor lines have been placed in tissue culture and an attempt is being made to correlate the presence or absence of A-particles with the production of specific immunoglobulins.

3. Recently studies have been initiated with Drs. Richard Asofsky and Mark Mandel to determine the differentiation of immunocompetent cells in virus-infected mouse systems. This has been possible because of techniques utilizing prolonged drainage of the thoracic duct. The cell types in the thoracic duct lymph have been classified on an ultrastructural basis and the differential cell count, covering a period of five days, has been made. These findings have been correlated with the in vitro production of immunoglobulins. Further efforts are being directed toward the fractionation and identification of the specific cells producing immunoglobulins.

4. A collaborative project with Drs. M. Fink and L. Sibal has been partially completed. This involved the study of the localization of viral antigens of Tween-ether treated Rauscher virus. The treated preparations were placed on a sucrose density gradient and the gradient fractions were evaluated by immunodiffusion, hemagglutination inhibition and electron microscopy. Preliminary results suggested the presence of antigens associated both with the nucleoid and the envelope.

5. Three projects are being done in collaboration with Dr. Padman Sarma. One involves the development of an in vitro system for the growth of the C-type particles associated with feline leukemia. Thymic extracts from leukemic kittens have been used to inoculate primary cultures of feline embryo fibroblasts and these cultures are monitored by electron microscopy for the presence or absence of budding particles.

Studies of the Novikoff hepatoma have revealed the presence of a C-type particle in the tumor, but attempts to propagate virus from this material have thus far been unsuccessful.

An extensive survey of spontaneous parakeet tumors has just been initiated. This includes EM studies for the detection of virus-like particles, testing for antigens common to known avian viruses, and establishment of tumor cells in tissue culture.

6. Monitoring activities were carried out with Dr. M. Chirigos on Sephadex column purified material used in establishing in vivo infections with the Friend leukemia virus. Samples from the infective fractions all contained C-type particles.

Significance to Biomedical Research and the Progress of the Institute:

The foregoing studies are in accord with the goals of the program in that they contribute information concerning the relationships of viruses to neoplasia and cell differentiation. Morphological classification continues as a primary parameter in establishing structure-function relationships. The studies of cell differentiation have established ultrastructural differences which were not apparent or suggested at the light microscope level. It is hoped that continued study of these animal model systems will contribute to a further understanding of the human problem.

Proposed Course of Project:

Most of the effort in the next fiscal year will be devoted to a continuation of the projects described herein. This section will continue to provide essential collaborative data to scientists in other sections and laboratories in the monitoring of various virus systems subjected to experimental design.

Publications

Fink, M. A., Herbut, P. A., Sibal, L. R., and Wivel, N. A.: "Probable Murine Origin of a Virus (HHLV-2) Recovered from Leukemic Mice After Inoculation of Human Leukemic Material." Journal of the National Cancer Institute, 39: 1253-1255, December, 1967.

Monroe, J., Shibley, G., Schidlovsky, G.,Nakai, T., Howatson, A., Wivel, N. A., and O'Connor, T.: "Action of Snake Venom on Rauscher Virus." Journal of the National Cancer Institute, 40: 135-145, January, 1968.

Kuff, E.L.; Wivel, N. A., and Lueders, Kira K.: "The Extraction of Intracisternal A-Particles from a Mouse Plasma-Cell Tumor." Cancer Research. (In Press).

Serial No. NCI-4828
1. Viral Leukemia & Lymphoma Branch
   OASDVO, Etiology
2. Virus & Disease Modification
   Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Modification of Oncogenic Viruses and Disease Induction by Immunological, Biological, Chemical, and Physical Methods.

Previous Serial Number: same

Principal Investigator: Michael A. Chirigos

Other Investigators:
Dr. William Hook
Dr. Willie Turner
Dr. Paul Ebert

Cooperating Units: Inside NIH:

Dr. J.B.Moloney, VLLB, NCI
Dr. K. Perk, VLLB, NCI
Dr. M.A.Fink, VLLB, NCI
Dr. L.R.Sibal, VLLB, NCI
Dr. T.O'Connor, VLLB, NCI
Dr. G. Canellos, Medicine Branch, NCI

Outisde NIH:

Dr. M. Gomez, Microbiological Assoc., Inc.,
Bethesda, Md.
Dr. G. Spahn, Microbiological Assoc., Inc.,
Walkersville, Md.
Dr. C. Burger, Dept. of Radiology
Upstate Medical Center
Syracuse, New York

Man Years:
Total: 5.0
Professional: 1.5 (one-half year devoted to Special Virus Leukemia program as Vice-Chairman of Special Animal Leukemia Ecology Segment, and Coordinator on Murine Leukemia Virus Production; Project Officer on 6 Contracts, and Asst. Project Officer on 8 contracts)

Other: 3.5

Project Description

Objectives:

The overall aim of this project is to determine whether oncogenic virus replication, primary virus-induced leukemias or lymphomas, or spontaneous leukemias or lymphomas can be effectively controlled. Several therapeutic approaches are examined in model animal systems. Any therapy leading to effective control is immediately exploited and, if feasible, submitted for preclinical testing and evaluation and eventual clinical trial. To obtain these objectives the project consists of three areas which are as follows:

1. To study the host-virus-cell interrelationships employing leukemogenic and sarcoma viruses, neoplasms induced by these viruses, spontaneous leukemias or lymphomas, and human tumor extracts containing virus-like particles.

2. To determine whether oncogenic virus replication, or primary virus-induced or spontaneous leukemias or lymphomas and sarcomas can be modified by biological, chemical, physical, or immune mechanisms, or a combination of these.

3. To investigate biochemical alterations which may occur in neoplastic tissues as a result of: oncogenic virus infection; chemical; or spontaneous induction.

Methods Employed:

A. Virus and Tumor Animal Systems:

1. The Rauscher, Friend, Moloney and Gross leukemogenic viruses, and the Moloney sarcoma virus, and our recent plasma passage sarcoma virus (SV-PP) variant are employed for *in vivo* and *in vitro* studies.

2. Transplantable tumor lines induced by Rauscher, Friend, and Moloney viruses as well as Moloney sarcoma and SV-PP are also employed *in vivo*.

3. AKR mice, which develop spontaneous lymphoma and a transplantable line of AKR lymphoma. SJL mice which have a high incidence of spontaneous reticulum-cell sarcoma.

4. Leukemia-sarcoma (RLV, FLV or MLV + MSV) virus-induced tumors and tumor extracts, which exhibit an increased oncogenicity in adult mice, are being employed as an aid in elucidating the murine leukemia-sarcoma complex *in vivo*.

5. The spleen foci assay system with the RLV and FLV, and a spleen colony assay system with our AKR lymphoma ascites cell line, have been established and quantitated for use in studies of anti-viral and anti-tumor therapy.

6. A gel filtration method has been developed for purifying leukemia virus from tissue homogenates. The method can be applied to preparing highly infectious and immunologically active materials.

7. *In vivo* and *in vitro* assay systems have been developed for interferon studies. Chemical and virus induced interferon produced *in vivo* is employed in *in vitro* mechanism of action studies.

8. Anti-mouse lymphocyte serum (AMLS). AMLS has been prepared in rabbits for *in vivo* therapy studies.

Major Findings:

1. Immune-Chemotherapy: Treatment of FLV or SV-PP (sarcoma) virus infected mice with homologous antibody at different stages of infection resulted in a significant reduction of infectivity titers and of splenomegaly (FLV) and tumors (SV-PP) with a concomitant increase in life span. The combined treatment with drug and antibody has been demonstrated to exert a more beneficial effect than either treatment alone. The increased therapeutic response with this combination is advantageous in that: (a) initial drug treatment, at subtoxic doses, arrests further neoplastic cell division and metastasis; (b) subsequent sero-therapy neutralizes extracellular virus thus inhibiting further viral transformation of cells to neoplasia; and (c) host immune-defense mechanisms and general health are not drastically impaired due to drug toxicity.

2. Immunological: Adoptive immuno-therapy, i.e.,treating immunosuppressed viremic animals with syngeneic spleen cells and lymphocytes obtained from vaccinated donors resulted in a limited therapeutic response. Most recent findings indicate that, rather than immunosuppressing animals by irradiation or drug treatment, carefully controlled treatment with anti-mouse lymphocyte rabbit serum may be more effective in preparing the host for treatment with antibody producing syngeneic lymphocytes.

3. Interferon: Two chemically defined agents, Statolon and divinyl ether maleic anhydride (a pyran copolymer) have been employed in interferon studies *in vivo*. By testing different regimens of treatment with these two agents, successful control of FLV has been achieved. Treatment with a low dose of the pyran copolymer daily for 5 days prior to FLV infection resulted in long-term survivors showing no evidence of disease.

4. Chemotherapy: The control of neoplasia (virus-induced or spontaneous) by drug therapy is continuing. Combination therapy (Vincristine, Cyclophosphamide and Prednisone) of AKR lymphoma has resulted in very effective control of the tumor. Significant increases in life-span have been effected by the combined treatment due to marked reduction in local tumor mass (complete regression) and hematologically disseminated disease. Deaths are considered to be due to causes other than leukemia, e.g. (secondary bacterial infection, irreversible metabolic or endocrine damage, renal or hepatic dysfunction, thrombocytopenia or anemia). Drugs are being administered based on body surface area rather than mg/kg, to make the information obtained more

meaningful for clinical application.

5. Test for presence of virus in biological materials (human, dog, cat, etc.): The spleen focus assay has been developed to the point where excellent quantitative and qualitative reproducibility has been achieved. The effect of different virus infections (Guaroa, vesicular stomatitis, mouse cytomegalo, adeno-, etc.) on FLV-induced spleen foci has been examined. Guaroa and VSV enhances in vivo replication of FLV as determined by an increased number of spleen foci, splenomegaly and FLV viremia. Cytomegalovirus and adenovirus did not appear to alter the expected number of FLV induced foci. Interferon induced de novo by drug stimulation markedly inhibits FLV-induced foci formation. This system is being used in assaying for low concentrations of virus. The observation that Guaroa virus at a dilution of 10^{-8} is capable of enhancing the number of FLV-induced foci indicates the sensitivity of this method to very low concentrations of virus. This system will be tested to determine whether a biological response (increase or decrease in foci formation, etc.) can be elicited as the result of infection with other virus containing biological materials (human, cow, cat, dog, etc.).

6. Virus-virus interactions: Regression of primary Moloney murine sarcoma virus-induced tumors were significantly altered by co-infection with low concentrations of leukemia viruses (Rauscher, Friend and Moloney). This effect was achieved with dilutions of RLV ranging from 10^{-1} to 10^{-5}. The increased incidence of tumor growth and deaths resulting from the dual infection is considered to be due to: (a) the leukemia virus acting as a "helper" for defective MSV resulting in the formation of a more virulent virus capable of inducing increased malignancy; (b) the immunological response of the host to MSV or MSV-specific antigens in emerging tumor cells may be altered by the infection with leukemia virus; or (c) a combination of both conditions. Intramuscular, intraperitoneal or subcutaneous inoculation of cell-free extracts, prepared from progressively growing tumors obtained from dually infected animals, results in rapid and extensive metastasis. Tumor nodules are observed in several tissues and organs which are not normally seen in animals inoculated with MSV within the observation period and, tumor regression does not occur. This increased oncogenicity has been maintained through four in vivo serial passages with cell-free tumor material. This evidence supports the first hypothesis (a). Employing the sheep RBC antigen assay it was shown that infection with RLV at dilutions of 10^{-1} to 10^{-4} results in a transient immunological suppression. This was not the case with the 10^{-5} dilution of RLV indicating that RLV may be acting as "helper" even at this low concentration. This evidence partially supports the second hypothesis (b). In contrast, reported in vitro studies have shown the requirement for a high concentration of leukemia virus for rescue of sarcoma genome.

Of particular significance is the high incidence of tumors induced in mice co-infected with RLV and or 10^{-5} dilution of MSV. This concentration of MSV does not elicit any detectable biological response when injected alone.

Significance to Biomedical Research and the Program of the Institute:

The multi-disciplinary approach represented in this project is almost entirely directed towards the prevention, treatment and control of leukemia. It is necessary to work with animal systems to gain basic information which ultimately will be applied to the control of human leukemia. The greatest progress in control of human leukemia has been by drug therapy which has several serious limitations. From animal model systems herein described, immunotherapy has proven to be an excellent control measure either alone or in combination with drug therapy. Other immuno-therapeutic applications are also worthy of further investigation (adoptive, anti-lymphocyte serum, etc.), and particularly the use of interferon. Sufficient therapeutic approaches are presently at hand, that if better understood and judicially applied, can serve to better control human leukemia.

Proposed Course of Project:

Each of the areas described under objectives will be pursued. More emphasis will be placed in refining the therapeutic measures already developed to the more efficient control of leukemia, lymphoma and sarcoma. Efforts will be made to further orient the application of therapy to the existing animal systems, so that the information gained from such studies can be applied clinically more rapidly and meaningfully.

Further *in vivo* studies will be conducted with the leukemia-sarcoma complex with the aim of understanding the mechanism of tumor induction by low virus input. Such studies may lead to a better understanding of the possible role of viral co-carcinogenesis in human leukemia and/or sarcoma.

The *in vivo* spleen focus forming assay system will be further refined to evaluate its usefulness as a diagnostic method in detecting the presence of virus in human leukemia and sarcoma material.

Honors or Awards: Dr. M.A.Chirigos presented, by invitation, a seminar on "The use of Murine Leukemia Animal Systems in the Development of Effective Therapeutic Measures Applicable to Human Leukemia" before the National Science Foundation, Science Trainees at Western Maryland College, on July 5, 1967.

Publications

Ebert, P.S., M.A.Chirigos, L.A.Fields, and P.A.Ellsworth.: Plasma Lactic Dehydrogenase and Spleen Heme Biosynthetic Activity Following Friend and Rauscher Leukemia Virus Infections. Life Sciences 6: 1963-1971, 1967.

March, R.W., Chirigos, M.A., Hook, W.A. and Burka, B.I.: Friend leukemogenic Virus-neutralizing Antibody from Mouse Ascitic Fluid. *Applied Microbiology*, 15: No. 6, 1414-1416, 1967.

Ebert, P.S., Chirigos, M.A., and Ellsworth, P.A.: Differential Response of Friend Leukemia Virus and Lactate Dehydrogenase Virus to Chemotherapy and *In Vitro* Neutralization. *Cancer Res.* 28: 363-367, 1968.

Chirigos, M.A., Scott, D., Turner, W., and Perk, K.: Biological, Pathological and Physical Characterization of a Possible Variant of a Murine Sarcoma Virus (Moloney). J. Internatl. Cancer, 3: March, 1968.

Turner, W., Chirigos, M.A., and Scott, D.: Enhancement of Friend and Rauscher Leukemia Virus Replication in Mice by Guaroa Virus. *Cancer Res.* 28: June, 1968.

Chirigos, M.A., Perk, K., Turner, W., Burka, B. and Gomez, M.: Increased Oncogenicity of the Murine Sarcoma Virus (Moloney) by Co-Infection with Murine Leukemia Virus. *Cancer Res*. 28: (June) 1968.

Serial No. NCI-4829
1. Viral Leukemia & Lymphoma Branch
   OASDVO, Etiology
2. Virus & Disease Modification
   Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Modification of Oncogenic Viruses and Disease Induction by Immunological, Biological, Chemical and Physical Methods.

Previous Serial Number: none

Principal Investigator: Dr. William A. Hook

Other Investigators:
Dr. Michael A. Chirigos
Dr. Willie Turner
Dr. Wilna Woods

Cooperating Units:

Inside NIH

Dr. J.B.Moloney
Dr. F.J.Rauscher
Dr. M.A.Fink
Dr. L.R.Sibal

Outside NIH

Miss S. Chan, Microbiological Associates, Inc., Bethesda, Maryland

Man Years:

Total: 1:0
Professional: 1.0
Other: 0

Project Description

Objectives:

The aim of this project is to determine whether oncogenic virus replication, primary virus-induced leukemias, lymphoma and sarcomas, or spontaneous lymphomas can be effectively controlled by immunological means. Model animal systems are used to examine experimental therapeutic approaches and any therapy demonstrating effective control is further investigated and, if feasible submitted for pre-clinical testing and eventual clinical trial.

Methods Employed:

A. Virus and Animal Systems:

1. The Rauscher (RLV), Friend (FLV) and Moloney (MLV) murine leukemia viruses were employed for *in vivo* studies. Effectiveness of immune

therapy was established by measurement of virus titer using the spleen weight assay, spleen foci count and by survival time.

2. The immunological activities of murine sarcoma virus (Moloney) [MSV(M)] and a possible variant derived from plasma (SV-PP) were also studied. The enhanced oncogenicity of MSV(M) observed in mice preinfected with RLV was demonstrated by measuring tumor area.

3. The naturally occurring lymphoma in the AKR strain of mice was studied by transplanting the tumor to recipients and preparing both cellular and cell-free vaccines. An attempt was made to modify the incidence and progression of the disease by immunization.

B. Immunological Approaches Tested:

1. Immunologic relationships between RLV, FLV, MLV, MSV(M) and SV-PP were studied *in vivo* by immunizing animals with formalin-treated vaccines and challenging with homologous and heterologous viruses. Studies were begun directed toward the development of a "polyvalent" vaccine capable of providing protection against a wide variety of oncogenic viruses.

2. Immunologic relationships between RLV, FLV, MLV, MSV(M) and SV-PP were also investigated by neutralization tests using antibodies prepared as mouse ascitic fluid.

3. Fieldsteel (personal communication) has suggested that human milk is capable of inhibiting FLV. To learn if such an effect might be demonstrable in the FLV neutralization test, fresh milks from 3 healthy donors, 1 cancer patient and a patient with a kidney infection were obtained and no significant degree of neutralization was observed.

4. Depressed levels of neutralizing antibody toward MSV(M) were demonstrated in mice preinfected with RLV. RLV was diluted to 10^{-2} and injected I.P. into 4-6 week old BALB/c mice 5 days before I.M. injection of MSV(M) diluted to $10^{-0.3}$. RLV and MSV(M) serum antibody neutralizing indexes, immune globulin levels and tumor size were followed to evaluate the effects of dual infection with these viruses.

5. Gel filtration was utilized to partially purify FLV from cell-free homogenates of infected mouse spleen. Formalin treated vaccine purified in this manner was tested for immunologic activity as antigen in complement fixation, immunofluorescence and animal protection tests.

Major Findings:

1. Cross Protection of Immunized Mice: Immunization with FLV afforded partial protection against challenge with FLV and RLV but not against MSV(M). RLV immunization afforded partial protection against challenge with FLV, and to a lesser degree against the homologous RLV. No important protection by RLV against MSV(M) was observed. Immunization with MLV gave complete protec-

tion against challenge with MSV(M) but little or none against RLV or FLV. MSV(M) immunization was capable of affording protection against MSV(M) challenge but little or no protection was observed vs. RLV or FLV infection.

2. Depressed Levels of Neutralizing Antibody in Dually Infected Mice: In adult BALB/c mice inoculated with MSV(M) a high percentage of tumor regressions occurred. Co-infection with RLV and MSV(M) resulted in progressively growing tumors, metastases and rapid deaths. This study showed that depressed levels of MSV neutralizing antibody were associated with progressive tumor growths. For example, sera from the dually infected mice obtained 42 days after MSV(M) infection had a neutralization index of 5, while a value of 214 was measured with serum from mice infected only with MSV(M). Enhancement of MSV(M)-induced tumors may be related to an immunosuppressive effect exerted by prior RLV infection. However, determination of IgG in the serum of the dually infected mice revealed levels slightly above those of MSV(M) or non-infected mice. The effect observed in these experiments may be due to RLV-induced immunosuppression. However, it cannot be explained by the absence of serum IgG.

3. Use of Gel Filtration to Purify Vaccine: Sephadex G-200 was employed to partially purify virus from FLV-infected spleens. Highly infectious and immunologically active material was eluted as a large initial protein peak while hemoglobin and other non-reactive materials were obtained in 2 subsequent peaks. The antigen fractions were reactive in the complement fixation (CF) and the fluorometrically quantitated, soluble antigen - fluorescent antibody (SAFA) tests with sera obtained from BALB/c mice either immunized, or infected with FLV. These antigen fractions exhibited cross reactions in CF and SAFA tests with antiserums to RLV, and to a lesser degree with antibody to MLV.

When crude vaccine was allowed to stand 21 days in the presence of 0.1% formalin and then passed through the Sephadex column a similar elution pattern was obtained. The material from the first peak of the formalin-treated vaccine was similarly reactive in CF and SAFA tests. Non-infectious antigen fractions of formalinized vaccine obtained from the first protein peak and combined with adjuvant were capable also of acting as immunizing antigen. Significant protection was achieved against a subsequent challenge with FLV diluted to 10^{-1}.

Significance to Biomedical Research and the Program of the Institute:

Studies described in this report contribute information likely to be of value to investigations involving human materials. Examples of cross-reactions among certain oncogenic viruses suggest immunologic relationships which may be of value in planning immunotherapy studies. The importance of immunosuppression to enhanced oncogenesis resulting from in vivo viral interactions is also indicated. Immunologic approaches to control of oncogenesis require sizeable amounts of highly active immunogens. A relatively simple method for obtaining partially purified antigen from virus infected spleen is described.

Proposed Course:

Continuation of research along the lines indicated is planned. Studies of vaccines will be extended, including development of a "polyvalent" preparation containing immunogens specific for several oncogenic viruses. Additional steps for the purification of vaccine are to be examined including other types of column separations and density gradient ultracentrifugation. Studies of the effects of immune suppression on oncogenesis will be continued and will involve other viruses and antilymphocyte serum.

Publications: March, R.W., Chirigos, M.A., Hook, W.A. and Burka, B.L.: Friend Leukemogenic Virus-neutralizing Antibody from Mouse Ascitic Fluid. Applied Microbiology, 15: 1414-1416, 1967.

Serial No. NCI-4830
1. Viral Leukemia & Lymphoma Branch, OASDVO, Etiology
2. Virus & Disease Modification Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Detection and Evaluation of Biochemical Alterations Occurring in Tissues or in the Intact Animal as a Result of Infection with Leukemia Virus and/or as a Result of the Induced Disease.

Previous Serial Number: same

Principal Investigator: Paul S. Ebert

Other Investigators: None

Cooperating Units: Inside NIH:

Dr. M.A.Chirigos
Dr. William Hook
Dr. Willie Turner
Dr. J.B.Moloney

Outside NIH:

Paul Ellsworth, Microbiological Assoc., Inc., Bethesda, Maryland

Man Years:

Total: 1.0
Professional: 1.0
Other: 0.0

Project Description

Objectives:

The biochemistry project involves the investigation of biochemical changes occurring in the tissues of animals inoculated with leukemogenic virus or tumor and the possible exploitation of these changes to modify or prevent the progression of the infection. The characteristics of the induction of δ-aminolevulinic acid (ALA) synthetase in spleens and livers of Friend (FLV) and Rauscher leukemia virus (RLV)-infected mice were investigated to determine whether or not these viruses are able to directly control activity in the heme biosynthetic pathway. Lysozyme activity in various organs of leukemia virus-infected mice was followed to determine if activity of this enzyme could be used as a sensitive measure of the progression of 3 viral leukemia infections. The activity of various enzymes of glycolysis and of 2 phosphate hydrolyzing enzymes in the Murine Sarcoma Virus (Moloney)(MSV)-induced rhabdomyosarcoma were investigated to determine certain characteristics of metabolism in order to provide a rational basis for chemotherapy of this tumor.

Methods Employed:

The following techniques were utilized:

1. The usual biochemical methods for the measurement of enzyme activities were followed.

2. Differential centrifugation was utilized to concentrate certain viruses for the determination of associated enzyme activity, and to determine enzyme activity in 5 subcellular fractions of normal muscle and MSV-induced rhabdomyosarcoma.

3. A column chromatographic technique reported by Tschudy, et al. in 1966 for the isolation and concentration of ALA from a tissue homogenate was utilized, refined, and adapted to mouse tissue.

4. Usual methods for the determination of hemoglobin, hematocrit, red blood cells, and reticulocytes were employed.

Major Findings:

A. δ-Aminolevulinic acid synthetase (ALA-synthetase) in FLV- and RLV-infected mice.

1. Delineation of optimal ALA synthetase activity in the spleens of FLV-infected mice was completed. Maximum enzyme activity occurred in a pH range of 7.0 to 7.2 and at an EDTA concentration of 10 mM when mice had been infected for 16 days. Other additives known to be necessary for optimum synthetase activity were tested for stimulatory effects on the FLV-spleen homogenate system.

2. ALA synthetase activity in spleen and liver of FLV-infected mice was followed and compared to rates of change of hematocrit, RBC, and hemoglobin levels. While the spleen weight during FLV infection increased 13 times the normal weight, the liver size remained essentially constant and the hemoglobin, hematocrit, and RBC level increased steadily for 20 days. On a gm tissue weight basis ALA synthetase activity remained 22-39% below normal for 14 days and thereafter attained normal or slightly greater than normal levels of enzyme. Only when the activity per spleen was calculated could an increase in enzymic activity be observed. Despite little or no change in the liver size, the enzyme activity per gram of tissue was decreased to 51% of the normal activity.

3. ALA synthetase was similarly followed during RLV infection. While spleen weight during RLV infection increased 33 times the normal weight, hemoglobin, hematocrit, and RBC levels decreased progressively

until the 23rd day. On a per gm tissue basis, splenic enzyme activity decreased by 40 to 57% of normal levels. When calculated on a per gm organ basis, activity increased progressively for 28 days due to the drastic increase in spleen size. Liver enzyme activity was decreased by 4 to 35% of normal activity expressed per gm liver, but showed a variable response on an organ basis since organ weight increased moderately during RLV infection. The large net increase in enzyme activity in the spleen in contrast to decreased activity in the liver emphasize the importance of the spleen as the prime erythropoietic organ in the mouse. Though no conclusion can be drawn regarding the possibility of a direct effect of the virus upon the RBC, it is likely the anemia promotes a compensatory response in ALA synthetase production. It was noted that as spleen hyperplasia increased, a relatively constant proportion of the tissue is responsible for ALA synthetase production.

4. Enzyme activity in the spleen and liver of phenylhydrazine-treated mice was investigated to observe whether or not chemically-induced anemia would mimic the effects of RLV. A similarity would help to determine the role of RLV in the altered erythropoiesis initiated by this virus. Spleen weight increased 4-fold, liver weight decreased slightly, and hematocrit dropped from 53% to 28% in phenylhydrazine-treated mice compared to normals. ALA synthetase activity in spleens of phenylhydrazine-treated mice increased to 240% of the normal level on a per gm basis and the activity in the liver was 81% of normal levels. These data emphasize the importance of mouse spleen in the control of erythropoiesis and show that the response of the spleen is somewhat different to RLV infection than it is to chemically induced anemia.

B. The lysozyme (muramidase) activity in plasma, spleen, and kidney was followed during the course of infection with FLV, RLV, and MLV. During MLV infection, the greatest increase in enzyme activity occurred in the plasma. Little change was noted in the spleen or the kidneys. As compared to normal lysozyme activity, enzyme activity in the plasma of FLV-infected mice was elevated for 28 days, activity in the spleen was consistently reduced for 50 days, and the activity in kidneys was markedly elevated until the termination of the experiment. The changes in the enzyme activity in the plasma, spleen, and kidney of RLV-infected mice were similar to the changes in the enzyme activity of the organs of mice infected with FLV.

C. Various aspects of carbohydrate metabolism in the MSV-induced rhabdomyosarcoma were examined. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are key enzymes in the hexosemonophosphate shunt pathway to produce ribose for RNA and DNA synthesis. 6-Phosphogluconate in the tumor attained 10-fold normal levels and glucose-6-phosphate dehydrogenase 40-fold the level in normal muscle 8 days after maximum size was reached. Both enzyme activities increased as the tumor enlarged and continued to increase as the tumor regressed. The activity of both enzymes in the tumor remained elevated above that found in normal muscle taken from the same respective site even 4 days after the tumor became non-palpable. Tumor glycogen was only 1/4 of normal muscle levels suggesting utilization of glycogen stores for energy and rapid growth. Fructose-1,6-diphosphatase, an enzyme in the glycolytic pathway controlling the resynthesis of glycogen, exhibited 40% less activity than did normal muscle.

D. Sarcoma virus-plasma passage (SV-PP) is a variant of MSV which exhibits a high virus titer in the plasma and induces splenomegaly in mice. As to be expected in animals infected with lactate dehydrogenase virus (LDV), these SV-PP-infected mice showed an LDV titer of $10^{6.6}$ 50% infectious doses/ml (ID_{50}/ml). Surprisingly, an MSV pool extracted from rhabdomyosarcoma tumors exhibited an LDV titer of $10^{8.2}$ ID_{50}/ml. This virus titer is higher than what would be expected in plasma 14 days after infection with LDV alone. This may indicate replication of LDV in the tumor.

E. In search of an enzymatic marker for SV-PP, ATPase activity of a 10-fold concentrated viral pellet was determined. Compared to an unwashed 10-times-concentrated pellet from normal plasma, an unwashed viral concentrate showed about 30 times the ATPase activity and a washed pellet about twice the activity. Subsequent experiments showed that only about one-half the virus was spun down at 105,000 x g. The centrifugation, however, did show 10 times as much ATPase activity in the pellet as was found in the supernatant solution. The data suggest that the viral pellet may contain ATPase activity.

F. Differential centrifugation experiments were conducted on virus-induced rhabdomyosarcoma and on normal muscle to compare the yields of subcellular fractions and to determine if the pathogenesis had any significant effect on the distribution of a soluble enzyme (LDH), a mitochondrial and microsomal enzyme (ATPase), and a lysosomal enzyme (acid phosphatase). In agreement with earlier experiments, the protein concentration of the tumor was reduced compared to normal muscle. The protein found in the nuclear fraction of tumor was only 22% of that found in the same fraction of normal muscle indicating a lack of synthesis of connective tissue in the tumor during enlargement. Also of interest are the greater than normal levels of protein occurring in the microsomal and supernatant fractions.

Rhabdomyosarcoma tumor homogenate exhibited 180% more acid phosphatase activity than an equivalent weight of normal muscle. The highest specific activities in both tissues occurred in the lysosomal fractions.

As was expected for a soluble enzyme, the highest activity was found in the supernatant fraction in both normal muscle and tumor. Both the homogenate and the supernatant fraction reflected a 75% decrease in the level of LDH in the tumor compared to normal muscle.

The ATPase activity of the tumor tissue was 47% less than that of the normal muscle. Though variable results were obtained with most fractions, the microsomal fraction of the tumor consistently demonstrated higher activity than did a like fraction from normal muscle.

Significance to Biomedical Research and the Program of the Institute:

The studies of the effects of FLV and RLV upon the control enzyme of the heme biosynthetic pathway are useful in delineating a possible role of viruses in directly controlling an important biochemical pathway involving the formation of blood elements. At present the RBC pathway is the only one sufficiently well described to be of use in determining an effect of a virus on a pathway at the molecular level. Data obtained in these investigations will be applicable to the possible role of viruses in the etiology of human erythroleukemia and to studies on erythropoietic porphria being carried on in the Metabolism Branch of the Institute.

Increases in lysozyme have been observed in cases of human monocytic and monomyelocytic leukemia. These studies have shown that lysozyme is the principal product of proliferating monocytes. The neoplastic cells in Friend disease have been variously designated as histiocytic, monocytic, or reticuloendothelial. Any increase of this enzyme in tissues of leukemic mice would have direct relevance to studies already conducted on human leukemic blood.

Studies on the enzymology and metabolism of the rhabdomyosarcoma induced by MSV have as their goal the formulation of a rational basis of therapeutic control based upon an understanding of the enzymic characterization of this tumor. This kind of study has an application to human muscle tumors since Freeman and Johnson have reported the similarity of the MSV-induced mouse rhabdomyosarcoma to rhabdomyosarcomas occurring in children as demonstrated by electron microscopy.

Proposed Course of Project:

The studies described in Objectives will be continued until more definitive results are obtained. The question regarding a direct or indirect effect of FLV or RLV upon the control enzyme of the heme biosynthetic pathway has not yet been resolved. A possible effect of the virus upon the half-life of both the messenger-RNA for the enzyme and ALA-synthetase will be pursued. Drugs and a hormone mimicing the effects of the 2 viruses on the enzyme will be tested in attempts to determine the mechanism of action of the viruses upon this pathway. Since enzyme activity is low and amount of tissue is strictly limited particularly in normal spleens of mice, attempts will be made to develop a micro method for ALA determination using radioactive and/or fluorimetric techniques.

Since the rhabdomyosarcoma induced by MSV exhibits unusual intracellular hydrogen transport, e.g., low LDH activity and reduced lactate accumulation compared to normal muscle, activity in the 2 remaining "shuttles" to provide NAD for glycolysis will be investigated to determine the general pattern of hydrogen transport. Once a pattern evolves, attempts will be made to direct chemotherapy at key enzymatic steps of metabolism.

Interrelationships between the LDH virus and MSV will be explored further.

The presence of ATPase activity in the pellet of SV-PP will be further investigated to determine if this parameter can be used as a marker for the virus and if this enzyme is part of the virion coat as was shown for the avian myeloblastosis virus.

Publications

Ebert, P.S., Chirigos, M.A., Fields, L.A., and Ellsworth, P.A.: Plasma Lactate Dehydrogenase and Spleen Heme Biosynthetic Activity Following Friend and Rauscher Leukemia Virus Infections. Life Sciences 6: 1963-1971, 1967.

Ebert, P.S., Chirigos, M.A., and Ellsworth, P.A.: Differential Response of Friend Leukemia Virus and Lactate Dehydrogenase Virus to Chemotherapy and *In Vitro* Neutralization. Cancer Res. 28: 363-367, 1968.

Serial No. NCI-4831
1. Viral Leukemia & Lymphoma Branch
   OASDVO, Etiology
2. Virus & Disease Modification Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Modification of Oncogenic Viruses and Disease Induction by Immunological, Biological, Chemical and Physical Methods.

Previous Serial Number: None

Principal Investigator: Willie Turner

Other Investigators:
Dr. Michael A. Chirigos
Dr. William Hook
Dr. Paul Ebert

Cooperating Units:

Inside NIH:

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Dr. K. Perk, VLLB, NCI
Dr. M.A.Fink, VLLB, NCI
Dr. L.R.Sibal, VLLB, NCI
Dr. T. O'Connor, VLLB, NCI
Dr. G. Canellos, Medicine Branch, NCI

Outside NIH:

Dr. M. Gomez, Microbiological Assoc., Inc., Bethesda, Md.
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Dr. C. Burger, Dept. of Radiology, Upstate Medical Center, Syracuse, New York

Man Years:

Total: 1.0
Professional: 1.0
Other: 0.0

Project Description

Objective:

The overall aim of this project is to elucidate, by in vitro and in vivo methods, interactions of oncogenic and non-oncogenic viruses. A better understanding of factors involved in such interactions would lead to more effective means of preventing or controlling oncogenic viruses.

Method Employed:

BALB/c mice were used for the *in vivo* studies reported. Conventional tissue culture method and standard virological techniques were employed in the *in vitro* studies undertaken in this report.

Major Findings:

Guaroa virus (GV), a member of the Bunyawera group of Arbor viruses, was shown to enhance FLV and RLV replication in mice. This enhanced replication of murine leukemia virus by prior infection of mice with GV was based on the fact that dually infected mice had enhanced spleen weight, spleen foci, and enhanced titers of recoverable virus from plasma and spleen when compared to animals inoculated with FLV or RLV alone. The enhancement of FLV or RLV replication induced by GV seemed to be dependent on the time of GV inoculation, since inoculation of GV as early as 6 days before FLV inoculation resulted in enhanced FLV replication, while inoculation of GV as early as 1 day after FLV infection did not result in enhanced FLV replication.

A dose response relationship existed between the dose of GV-inoculated and the magnitude of enhancement of FLV replication. A dose of GV as low as 100 $SMLD_{50}$ was able to significantly enhance FLV replication.

Studies were conducted to determine the mechanism by which GV induced the enhancement of FLV and RLV replication in dually infected mice. The possibility was considered that GV could act as an immunosuppressor or could suppress interferon synthesis. Experiments were undertaken to determine the effect of GV infection on the ability of NDV to produce interferon in mice, and, to inhibit antibody production against sheep erythrocytes. Results obtained indicated that GV did not function as an immunosuppressor in the mouse nor did the virus alter the ability of mice to synthesize interferon to NDV. Thus the mechanism by which GV enhances murine leukemia virus replication in mice is not known. Experiments are now underway to investigate whether GV induced enhancement of FLV and RLV replication in mice may be due to an increase in leukemia virus susceptible spleen cells mediated by GV infection.

VSV(RNA) Adenovirus type 12 (DNA), and mouse cytomegalovirus (DNA) were tested for their ability to enhance FLV replication in mice. Only VSV was shown to enhance replication of FLV.

In summary, data obtained indicated that GV (RNA) or VSV (RNA), enhances replication of FLV and RLV *in vivo*; (2) prior inoculation of GV, or inoculation of GV at the time of FLV or RLV inoculation is necessary for potentiation of enhanced replication of FLV or RLV; (3) infectious GV seems to be necessary for the enhanced replication of FLV and RLV as indicated by the dose response relationship between dose of GV inoculated and the magnitude of enhancement of RLV or FLV replication in dually infected mice; and (4) the underlying mechanism by which GV enhances FLV and RLV replication in mice is not due to the ability of GV to act as an immunosuppressor or to shut off the interferon synthesizing mechanism of mice.

Murine Leukemia Virus + Moloney Sarcoma Virus interaction in vivo:

Studies were undertaken to determine the effect of murine leukemia virus infection on MSV[M] oncogenicity *in vivo*. Results indicated that mice dually infected with FLV + MSV, RLV + MSV, and MLV + MSV showed a higher percentage of progressively growing tumor and a higher percentage of death with tumors than mice infected with MSV alone.

Mice infected with RLV as early as 5 days prior to, or 6 days after MSV infection develop tumors with progressive growth and show an enhanced mortality with tumor as compared to mice inoculated with MSV alone.

The enhanced incidence of mortality with non-regressing tumors in mice dually infected with RLV and MSV showed a dose response relationship with dilution of RLV from 10^{-2} and 10^{-5}. Mice inoculated with a dilution of RLV as high as 10^{-5} and subsequently inoculated with MSV exhibited only 10% regression of tumor as compared to 70% regression of tumor in mice infected with MSV alone. Mice inoculated with a constant dilution of RLV (10^{-2}) 5 days prior to inoculation of varying dilutions of MSV (10^{0} to 10^{-5}) showed 20% tumor induction with a dilution of MSV as high as 10^{-5}. This dilution (10^{-5}) of MSV when inoculated alone in mice produced no tumors.

In summary, results of these studies indicated that; (1) dual infection of mice with MSV and RLV, FLV, or MLV, enhances oncogenicity of MSV; (2) the RLV potentiated enhancement of MSV oncogenicity can be produced by inoculation of mice with RLV as early as 5 days prior to, and as late as 6 days after MSV inoculation; (3) a dose response relationship exists between the dose of RLV inoculated and the magnitude of enhancement of MSV oncogenicity, and (4) inoculation of mice with RLV can be used to demonstrate MSV activity in a dilution of MSV (10^{-5}) below the endpoint of the MSV pool.

These results seem to suggest that RLV and other murine leukemia viruses (FLV, MLV) act as "helper" virus for the defective MSV *in vivo*.

Interaction of Guaroa Virus (GV) and MSV in vitro and in vivo:

Studies have been undertaken to determine the effect of GV on MSV oncogenicity *in vitro* and *in vivo*. Results obtained indicate that prior inoculation of GV enhances MSV oncogenicity. This is indicated by the enhanced percentage of death with tumor, reduction in median survival time, and increased size of tumors in mice dually infected with GV and MSV when compared with those infected with MSV alone.

Attempts to show that GV enhances focus formation in mouse embryo fibroblast (MEF) culture infected with GV + MSV have been equivocal. Attempts to recover MSV activity from the fluid of MEF culture dually infected with GV + MSV have been unsuccessful.

Experiments were also undertaken to rescue the MSV genome from a non-producer line of hamster cells (Messore line) transformed by MSV by co-cultivation of hamster cells and MEF in the presence of GV. Bioassay of fluid from these cultures in mice showed no MSV oncogenic activity.

Reactivation of MSV tumor activity in mice with regressed MSV-induced primary tumor by inoculation of RLV:

Studies on the ability of RLV to reactivate MSV oncogenicity in mice when the primary MSV induced tumors had regressed were undertaken. Results of these studies show that an enhanced appearance of MSV tumors occurred in mice which were free of the primary MSV induced tumor by infection with RLV. Experiments are currently in progress to further elucidate this phenomenon.

The effects of sonication on FLV, RLV, and MSV.

Data on the effects of sonication on murine leukemia and sarcoma viruses indicate that Moloney sarcoma virus is more resistant to sonication than either FLV or RLV. In 5 minutes of sonication at full capacity in a Bromwell sonicator, complete destruction of FLV and RLV infectivity occurred, but only partial reduction of MSV was achieved. This observation is being pursued for more quantitative data for it may provide a method of separating murine leukemia virus from MSV pools.

Significance to Biomedical Research and the program of the institute:

A current theory of carcinogenesis in man implicates virus as a promoting factor (co-carcinogenesis). The virus or promoting factor may be dormant until triggered either by trauma, chemicals or a virus infection. Thus the current studies involving virus interactions will lead to an understanding of co-carcinogenesis that may occur in man.

Proposed Course:

Studies on interactions of oncogenic and non-oncogenic viruses will be continued. The major direction of these studies will be toward elucidating the mechanism by which the observed enhancement phenomenon was induced. The studies will also be extended to determine the effects of other viruses (non-oncogenic) on replication of murine leukemia and sarcoma viruses *in vitro* and *in vivo*.

Publications

Turner, W., Chirigos, M.A. and Scott, D. Enhancement of RLV and FLV replication *in vivo* by Guaroa virus. Cancer Res., June, 1968.

Chirigos, M.A., Perk., K., Turner, W., Burka, B., and Gomez, G. Effects of co-infection of mice with murine leukemia virus and MSV on MSV oncogenicity. Cancer Res., June, 1968.

Chirigos, M.A., Scott, D., Perk., K., and Turner, W. Biological and physical characterization of a possible variant or Moloney sarcoma virus. Internatl. J. of Cancer, June, 1968.

Serial No. NCI-4815
1. Viral Leukemia and Lymphoma Branch
OASDVO
2. Viral Leukemia Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: (A) Human Leukemia Section
(B) *In Vivo* and *In Vitro* Studies with a Virus-Induced Sarcoma
(C) *In Vitro* Studies with Human Leukemias and Sarcomas

Previous Serial Number: NCI-4879

Principal Investigator: Dr. Michael V. Viola

Other Investigators: None

Cooperating Units: (A) Dr. Clark W. Heath, Jr., National Communicable Disease Center, Atlanta, Georgia
Dr. Peter McPhedran, NCDC
Dr. D. Clemont, Yale-New Haven Medical Center
Dr. Louis Leone, Rhode Island Hospital
Dr. John Rouse, CDC, Kansas City
Dr. A. Moon, Medical College of Virginia
Dr. N. Weisberger, Western Reserve Medical School, Cleveland, Ohio

(B) Dr. Kirby Smith, NCI, VLLB
Dr. Nelson Wivel, NCI, VLLB
Mr. G. Shidlowsky, Charles Pfizer & Co, Maywood, New Jersey
Dr. Steward Finch, Yale-New Haven Hospital
(C) Dr. Hugh Butt, Mayo Clinic

Man Years:
Total: 5
Professional: 1
Other: 4

Project Description

Objectives:

(A) To collect potentially biologically active specimens from selected human leukemia patients and to investigate human leukemia clusters on time and space and to study human-animal tumor associations.

(B) 1. To determine the serological response of mice to sarcoma virus infection with emphasis on the tumor induction and regression phases.

2. To compare the transplantation antigens of primary Moloney sarcoma virus (MSV)-induced tumor and its metastases and to determine the role of humoral and cellular factors in transplantation resistance within this system.

3. To characterize a morphological variant of the MSV.

4. To determine if granulocyte and monocyte kinetics are altered in MSV-induced leukemia.

(C) 1. To procure human sarcoma tissue and establish culture cell lines for future virus and/or genome rescue work.

2. To examine by electron microscopy polyps from patients with familial polyposis and to establish cell lines from those specimens.

Methods Employed and Major Findings:

(A) Human Leukemia Studies: In July 1967, a family from Naugatuck, Connecticut was interviewed and plasma obtained at Yale-New Haven Hospital. There are four siblings in the family. One child (10yr. male) has acute lymphocytic leukemia. He has a non-idenical twin. No virus particles were seen in plasma pellets taken from family members. Chromosome studies were normal.

In August 1967, two families were interviewed in Beulah, N.D. Two adult males who lived next door to each other developed acute monocytic leukemia within the same month. Both lived in Beulah all their lives. Radiation levels were normal in that area and no chemical exposure could be determined. Neither had virus particles in their plasma. Chromosome studies were normal. Long-term lymphocyte cultures were established from both patients.

In September, 1967 a family was interviewed at the Balto. Public Health Hospital. The father had acute myelogenous leukemia of 2 mos. duration. His two year old child had a rhabdomyosarcoma removed from his deltoid muscle. No other pertinent familial data was obtained. The father had no virus-particles in his plasma pellet. No specimens were available from the child.

In January 1968, a family was interviewed in Richmond, Va. A 4-yr. old boy had acute lymphoblastic leukemia (ALL). No other siblings were in the family but the patient has lived with a 6-yr. old female cousin who died in Nov. 1965 with ALL. A paternal second cousin also had ALL. No virus particles were seen in plasma from parents and child. Lymphocyte cultures were established on all three. Chromosome studies were normal. Further studies on other family members are in progress.

In February 1968, an adult patient with lymphosarcoma was interviewed in Cleveland, Ohio. His dog was sacrificed and found to have lymphosarcoma. Electron microscopy studies of the pets' plasma, liver, spleen and lymph nodes were negative for virus-like particles. This case is the sixth patient-pet association we have documented.

(B) Serological and transplantation studies with the Moloney Sarcoma Virus system:

1. The effect of incubating MSV-induced mouse tumor with immune sera and immune lymphocytes was determined. Both had an inhibiting effect on the growth of transplanted tumors but immune lymphocytes were more effective. The degree of tumor suppression by antisera was related to the dilution. Incubation of tumor cells with antisera in the presence of lymphocytes produced greater tumor suppression than either alone.

2. Adult mice inoculated with high dilutions of MSV develop tumors which eventually regress. Many of these animals later die of widespread metastases in the absence of tumor at the site of inoculation. It was found that the metastatic cells had a faster growth rate than primary tumor cells in isogeneic animals. Similarly, metastatic cells appeared less antigenic because of lack of growth suppression by immune lymphocytes and their ability to grow in immune animals.

3. The serological response to MSV induction and regression was studied by *in vitro* focus formation neutralization and complement fixation. Trypsinized or mechanically disrupted mouse or hamster sarcoma cells were not susceptible to cytolysis by antisera. A cytotoxic test using an ascites variant of the sarcoma has now been developed. Because of the low *in vitro* neutralization titres obtained comparison of *in vitro* focus formation neutralization and the *in vivo* neutralization of oncogenicity is now in progress. Also, animals rejected challenge with MSV tumor cells up to three months after regression of their primary tumor.

To determine if the tumor cell directed antibody is different from the virus directed antibody, mice were immunized with virus free, CF negative hamster tumor cells; normal hamster muscle was used as a control. The serological response and transplantation resistance of these animals is presently being studied.

4. Electron Microscopic Studies: Electron microscopic examination of the rapid passage MSV-induced mouse tumor was performed. Recent passages showed marked pleomorphism of MSV in contrast to previous reports of a consistent "C" type morphology. Tumors were free of most murine virus contaminants including pneumonitis virus of mice and Sendai virus which replicate in a manner similar to that observed for the pleomorphic particle.

5. Previous work in this laboratory has shown that a lymphatic leukemia inducing agent (Moloney Leukemia Virus) can induce profound changes in the red blood cell kinetics of recipient mice. Serum and organ lysozyme levels of mice inoculated with the same virus were done at different stages of infection to determine if granulocyte and monocyte kinetics were also altered. No elevation in lysozyme levels was found.

(C) Collection of human sarcomas from various hospitals was continued. Tumors were grown in tissue culture and then stored in liquid nitrogen when cultures were established. Electron microscopic examination (carried out at Charles Pfizer & Co.) of tumor cells in culture have been negative for virus-like particles. Sarcoma genome rescue experiments have been attempted by co-cultivation of tumor cells with normal cells (human amnion, human embryonic lung, monkey kidney)followed by infecting cultures with a number of potential "helper" viruses (influenza, parainfluenza, reo-virus 3, concentrated human leukemic plasma with "C" type particles). Supernatant from infected cultures were assayed on a number of cell lines. No focus-forming or cell-transforming activity was found.

A project was begun with Dr. Hugh Butt (Mayo Clinic) for the electron microscopic examination and tissue culture study of polyps from patients with familial polyposis (a premalignant lesion). No particles were seen in the first specimen received.

Significance of Biomedical Research and the Program of the Institute:

The epidemiological significance of temporal-spatial clustering of leukemia and patient-pet tumor associations is still unresolved. However, it is imperative that all such occurrences be studied from a virological aspect with the tools presently available. Sera is being stored from all cases investigated for future use when an applicable serological test becomes available.

The Moloney Sarcoma Virus system is an excellent model for the study of tumor rejection mechanisms in the isogeneic host and subsequent metastatic survival in an immune host. The masking, altering or deletion of metastatic cell antigens necessary for tumor cell survival has many implications in human oncology. The different components involved in rejection of an RNA-virus-induced tumor (MSV) as well as attempting to distinguish new cellular tumor antigens from virus antigens will provide an important comparison with the DNA-tumor virus system.

The finding of pleomorphic virus forms raises the possibility of an incompetent virion (as is the case in pleomorphic influenza forms) or a totally competent (i.e. leukemia-sarcoma hybrid) virion. This work will complement *in vitro* studies of MSV in the Viral Biology Branch.

Proposed Course:

Further studies include the serological investigation and investigation of animals immunized with virus-free MSV tumor (hamster).

The attempted separation of pleomorphic MSV forms (by tissue culture passage and biophysical methods) will then enable us to biologically characterize this particle.

Publications

Viola, Michael, V.: Acute Leukemia and Infection. Journal of the American Medical Association, September 18, 1967, Vol. 201, pp. 923-926.

Viola, M. V., Dalton, A. J., Mitchell, E., Moloney, J. B.: Virus-like Particles in a Patient with Chronic Lymphocytic Leukemia. New England Journal of Medicine. 277: 503-506, September 7, 1967.

Viola, Michael V. and Moloney, John B.: Transplantation Antigens of the Moloney Virus-Induced Sarcoma and its Metastases. Submitted to the Journal of the National Cancer Institute, March,1968.

Viola, Michael, V.: The Occurrence of Hematological Malignancies in Patients and Their Pets. Submitted to the Journal of the American Medical Association, April, 1968.

Serial No. NCI-4816
1. Viral Leukemia & Lymphoma Branch
AOSDVO, Etiology
2. Viral Leukemia Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: (A) Study of a filterable agent isolated from Sarcoma 37.
(B) Studies on the induction of plasma cell tumors in mice.
(C) In Vivo histopathology of mouse tumors.

Previous Serial Number: NCI-4877

Principal Investigator: Dr. Ruth Merwin

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	4
Professicnal:	1
Other:	3

Project Description

Objectives:

(A) Further studies of filterable agent(s) isolated from Sarcoma 37.

(B) To continue studies on the pathogenesis of plasma cell tumors induced by plastic discs.

(C) The in vivo observation of the microcirculation of normal and leukemic mice.

Methods Employed and Major Findings:

(A) 1. Cell-free filtrates of Sarcoma 37 were passed in mice. It was found that continued passage of spleen derived filtrate caused non-neoplastic spleen enlargement whereas passage of lymph node derived material caused mostly lymphoid tumors. An agent which induces bone lesions was also isolated.

2. When Sarcoma 37 filtrate was neutralized with Moloney leukemia virus antiserum, a reduction in bone lesions was observed.

3. Sarcoma 37 filtrates were inoculated repeatedly in Balb/c mice and then organ filtrates introduced into other strains of mice. It was noted that the biological characteristics of the filtrate could be changed by introduction into a foreign strain of mouse.

(B) It was found that the introduction of plastic discs intraperitoneally caused fewer plasma cell tumors in mice than was previously reported. Various degrees of mechanical trauma to the abdomen was found to have no effect on the incidence of plastic disc induced plasma cell tumors.

(C) Visualization of the microcirculation of selected rodents was obtained by implanting 2 small panes of glass 5-10 micro thick subcutaneously. Glass was wired together because no adequate adhesive was found. Tone fibers were used as buffers between the panes of glass and embryonic tissues were placed adjacent to the window to provide a good vascular supply. Studies are now in progress comparing the microcirculation of leukemic and normal mice.

Significance to Biomedical Research and the Program of the Institute:

(A) Study of a bone lesion inducing agent from Sarcoma 37 is of interest because of the extensive work with the Moloney leukemia agent also isolated from S-37. The S-37 agent does not induce thymic lesions as does MLV. The study of this agent may aid in determining to what extent the MLV is a mixture of different agents, a mixture of variants or a mixture of modulated forms.

(B) These studies are important in contributing to a further understanding of the pathogenesis of plasma cell tumors.

(C) Studies of the circulation at the capillary level will contribute to an understanding of the basic factors controlling the flow of blood in fine vessels and this knowledge should be useful in understanding the changes in circulation that occur in animals with leukemia or in leukemic animals treated with various chemotherapeutic agents.

Proposed Course:

(A) Future studies will include the determination of the various components of our S-37 filtrates, e.g. the identification of known leukemia viruses.

(B) Future studies will determine whether the incidence of plastic induced plasma cell tumors has a relation to the numbers and types of bacterial and viral infections common in Balb/c mice colonies. The long term experiments now in progress on the induction of fibrosarcomas will be continued.

(C) Cinematography of the microcirculation of normal and leukemic mice will be attempted.

Serial No. NCI-4817
1. Viral Leukemia & Lymphoma Branch
OASDVO, Etiology
2. Viral Leukemia Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1967 through June 30, 1968

Project Title: Studies on the Moloney Sarcoma Virus (MSV)

Previous Serial Number: None

Principal Investigator: Kirby L. Smith

Other Investigators: None

Cooperating Units: Staff, Viral Leukemia Section

Man Years:

Total:	4.5
Professional:	1.0
Other:	3.5

Project Description

Objectives:

(A) To quantitate viral replication during induction, regression, and recurrence of MSV-infected Balb/c mice.

(B) To correlate levels of circulating antibodies with regression and recurrence of MSV-induced tumors in Balb/c mice.

(C) To continue studies on the host range of MSV.

Methods Employed and Major Findings:

(A) Viral replication in MSV-infected Balb/c mice. Previous reports from this and other laboratories have suggested that MSV replicates not only in tumor tissue but in other organs as well. Studies have been instituted to quantitate virus multiplication during the various stages of tumor induction and regression by the *in vitro* focus assay method.

Data accumulated to date from animals infected at 2 weeks of age shows that measurable titers of virus appear at the inoculation site at 3-4 days post inoculation and reach maximum titers at 7-10 days. Preliminary results suggest that tumor material 4-5 days after inoculation contains a defective component but by 7-10 days appears competent. One week after infection $10^{1.7}$ FFU/ml are detectable in plasma. Two weeks after infection $10^{3.7}$ FFU/ml are detectable in spleen and $10^{2.95}$ FFU/ml in liver. Even after complete tumor regression as much as 10^{4} FFU/ml of virus are present at the inocculation site and organs including spleen, liver and lungs contain 10^{2}- 10^{4} FFU/ml of virus.

Pilot studies have been initiated to determine the effect of splenectomy on viral replication in Balb/c mice. Early data suggests that the latent period for tumor development is longer, tumor growth occurs slowly, however, time to death is shorter than in comparable intact animals. Studies are now underway to quantitate viral multiplication in these animals.

(B) Circulating antibodies during regression and recurrence of MSV-induced tumors in Balb/c mice.

In order to determine whether a correlation exists between humoral antibody levels and growth of the sarcoma, serum levels are being tested at various stages of tumor development. Complement fixing antibody titers are being assayed according to the method of Hartley. Early data has demonstrated titers of 1:4 to 1:32 during the period of regression, but further study is necessary to determine whether positive titers are a constant finding and do correlate with the stage of tumor growth.

(C) Further studies on the host range of MSV. MSV has been shown to be oncogenic for mice, rats and hamsters. *In vitro* studies have demonstrated transformed cellular morphology in infected mouse and rat embryo cells. Studies in this laboratory are underway to demonstrate the ability of MSV to transform hamster embryo cells *in vitro*. The necessity for high titer virus stocks is anticipated and 10^{7} FFU/ml have been achieved by further partial purification and concentration of mouse tumor material. To date no transformation has been observed using high titer mouse and rat derived virus, various adsorption periods, various maintenance medias for cells infected in monolayer and in suspension. The induction of CF antigen in MSV-infected cells has not been observed.

Further studies on host range of MSV have included attempts to induce tumors in primates with cell free virus and whole cell mouse tumors. Rhesus monkeys observed over a 2-3 year period have remained tumor free.

Recent work with Rous Sarcoma Virus has shown that certain strains appear to develop enhanced oncogenicity for monkeys by passage first through monkey-derived tissue culture cells. With this study as a model, work is now underway to extend the host range of MSV to monkeys by passage through monkey tissue culture. Preliminary work using embryonic Rhesus muscle, spleen and kidney cells has been carried out.

No altered cell morphology or CF antigen induction has been demonstrated. Further study is in progress using high titer virus stocks in primary and continuous monkey derived tissue culture cells.

Significance to Biomedical Research and Program of the Institute:

The murine sarcoma-leukemia system continues to be a prime model system for the study of viral oncogenesis. Extension of knowledge of the virus-host interaction opens new channels for investigation for the possible viral etiology in certain human malignancies.

Proposed Course:

(A) Studies on viral replication will be continued and include adult mice, young and adult rats in an attempt to delineate host factors and innate properties of the virus for multiplication.

(B) Further studies on the development of CF antibody and neutralizing antibody will be continued. Attempts to develop a cytotoxicity test for the sarcoma system will be carried out in order to study cytotoxic antibody development.

(C) Continued studies on host range of MSV *in vivo* and *in vitro*.

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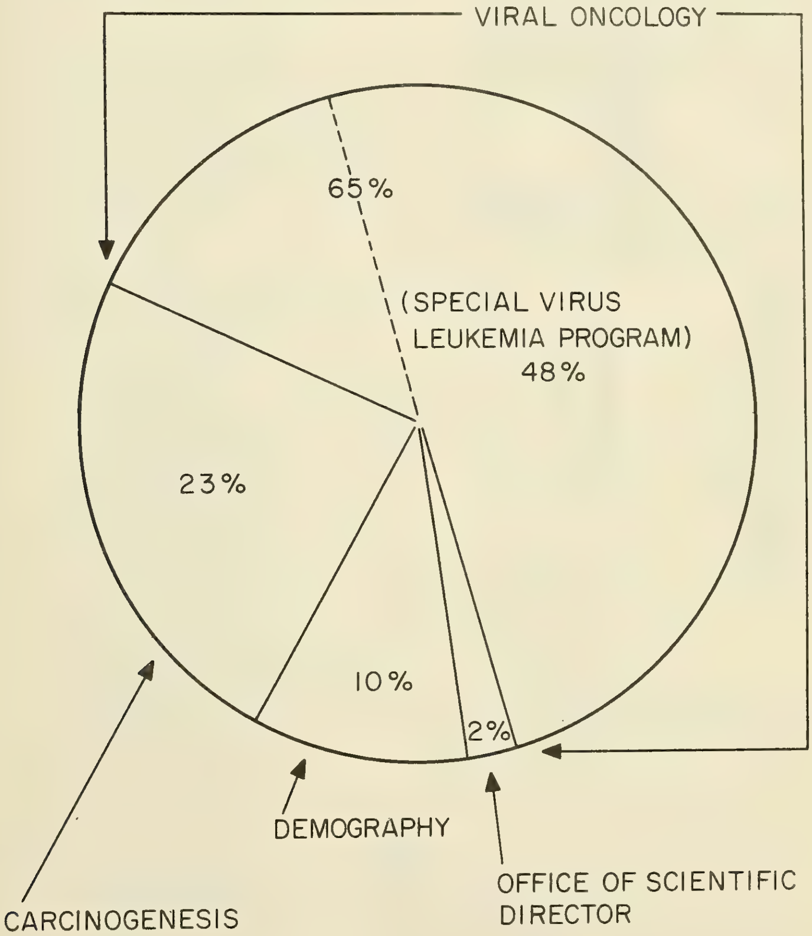

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TABLE OF RATIOS OF CONTRACT TO INTRAMURAL EFFORT BASED ON CURRENT LEVEL OF EXPENDITURE
JUNE 30, 1968

	OSDE	Demography	Carcinogenesis	Viral Oncology		Total
				Basic Program	Special Virus Leukemia Program	
Total	609,000	3,056,000	7,238,000	5,559,000	14,996,000	31,458,000
Contracts	---	1,850,000	5,602,000	3,928,000[3]	14,287,000[4]	25,667,000
Intramural*	609,000[1]	1,206,000	1,636,000	1,631,000[2]	709,000[4]	5,791,000
Ratios: Contract to Intramural	---	1.5:1	3.4:1	2.4:1	20:1	4.4:1

* Includes costs of managing contract activities.

[1] Includes $258,000 for data processing for all Branches.

[2] Includes $153,000 in support of NIAID Intramural Operations.

[3] Includes $275,000 funded by Breast Cancer Task Force.

[4] Excludes Therapy portion of Special Virus Leukemia Program ($2,045,000).

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